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TITLE OF THE INVENTION (280 characters max)

TGFβ-1 SUPPORTED AND BINDING PEPTIDES

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ENCLOSED APPLICATION PARTS (*check all that apply*)

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Respectfully submitted,

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Date: December 19, 2003 By: Kay L. Gavaglio

PROVISIONAL PATENT
ATTORNEY DOCKET NO. GC826P

TGFβ-1 SUPPORTED AND BINDING PEPTIDES

This application claims priority to U. S. Provisional Application 60/518,154, filed November 3, 2003, and U.S. Provisional Application 60/520,403 filed November 13, 2003, which are hereby incorporated by reference.

Field of the invention

The invention is directed to peptides and supported peptides that bind TGFβ-1.

Background of the invention

Proteins of the Transforming Growth Factor-β (TGFβ) family are synthesized by almost all cells. The TGFβs are a group of stable, multifunctional polypeptide growth factors whose activities include, among other things, context specific inhibition and stimulation of cell proliferation, control of the extracellular matrix and degradation and control of mesenchymal-epithelial interactions during embryogenesis, mediation of cell and tissue responses to injury, control of carcinogenesis and modulation of immune responses. Structurally, each TGFβ monomer consists of two anti-parallel pairs of β strands which form a flat curved surface, a separate long alpha-helix and a disulfide-rich core with a cysteine knot (*see,* S. Daopin, M. Li, & D.R. Davies (1993). Proteins, Structure Function and Genetics, 17:176-192. Crystal structure of TGFß2 refined at 1.8 Å resolution; S. Daopin,K.A. Piez, Y. Ogawa, & D.R. Davies (1992). Science, 257:369-373. Crystal structure of transforming growth factor-ß2: An unusual fold for the superfamily and S.Daopin, G.H. Cohen, & D. Davies (1992). Science, 258:1160-1162.Structural similarity between transforming growth factor-ß2 and nerve growth factor : response)). These growth factors are secreted as latent pro-TGFβs, and the C-terminal is cleaved by a subtilisn-like pro-protein convertase protease to form TGFβ. Most TGFβs form disulfide-linked homodimers and signal via serine/threonine receptor complexes.

TGFβ-1 is synthesized, with only a few exceptions, by virtually all cells. TGFβ-1 has been found in the highest concentration in human platelets and mammalian bone. TGFβ-1 has many functions including suppression of cell proliferation, enhancement of extracellular matrix deposition and physiological immunosuppression. TGFβ-1 has also been determined to be biologically active in hair follicle development. Human TGFβ-1 is a 25.0 kDa protein with subunits that contain approximately 112 amino acids per subunit. Two different receptor proteins are involved in TGFβ-1 binding and signalling, TGF-RβII and TGF-RβI.

The Bowman-Birk protease inhibitor (BBI) is a designation of a family of stable low molecular weight trypsin and chymotrypsin enzyme inhibitors found in soybeans and various other seeds, mainly leguminous seeds and vegetable materials. BBI is a family of disulfide bonded proteins with a molecular weight of about 8 kD. (Chou et al. (1974) Proc. Natl. Acad. Sci. USA 71:1748-1752; Yavelow et al. (1985) Proc. Natl. Acad. Sci. USA 82:5395-5399; and Yavelow et al. (1983) Cancer Res. (Suppl.) 43:2454s-2459s). BBI has a pseudo-symmetrical structure of two tricyclic domains each containing an independent native binding loop, the native loops containing binding sites for both trypsin and chymotrypsin (Liener, I. E., in R. J. Summerfield and A. H. Bunting (eds), Advances in Legume Science, Royal Bot. Gardens, Kew, England). These binding sites each have a canonical loop structure, which is a motif found in a variety of serine proteinase inhibitors (Bode & Huber, Eur. J. Biochem. (1992) 204:433-451). Commonly, as in one of the soybean inhibitors, one of the native loops inhibits trypsin and the other inhibits chymotrypsin (Chen et al., J. Biol. Chem. (1992) 267:1990-1994; Werner & Wemmer, 1992; Lin et al., Eur. J. Biochem. (1993) 212:549-555; Voss et al., Eur. J. Biochem. (1996) 242:122-131) though in other organisms (e.g., Arabidopsis), both loops are specific for trypsin.

The Kunitz-type soybean trypsin inhbitor (STI) is another protease inhibitor that inhibits the proteolytic activity of trypsin by the formation of a stable stoichiometric complex. (*See*, e.g., Liu, K., Chemistry and Nutritional value of soybean components. In: Soybeans, chemistry, technology and utilization. pp. 32-35 (Aspen publishers, Inc., Gaithersburg, Md., 1999)). STI consists of 181 amino acid residues with two disulfide bridges and is roughly spherically shaped. (See, e.g., Song et al., J. Mol. Biol. 275:347-63

(1998)). The two disulfide bridges form two native binding loops similar to those described below for BBI.

Eglin C is a small monomeric protein that belongs to the potato chymotrypsin inhibitor family of serine protease inhibitors. The proteins that belong to this family are usually small (60-90 amino acid residues in length) and contain no disulfide bonds. Eglin C, however, is highly resistant to denaturation by acidification or heat regardless of the lack of disulfide bonds to help stabilize its tertiary structure. The protein occurs naturally in the leech *Hirudo medicinalis*.

Formation of hair follicles involves a complex series of steps: growth (anagen), regression (catagen), rest (telogen) and shedding (exogen) (Stenn, K.S., and Paus (2001). Physiological Reviews, Vol 81, No. 1, Controls of Hair Follicle Cycling). TGFβs have been implicated as one of the major drivers of the transition from anagen to catagen in the hair cycle (Foitzik, K et al, 2000, FASEB and Soma, T., et al 2002, JID). Conditional TGFβ-1 expression in transgenic mice demonstrates that one can induce alopecia reversibly (*see* Lia, X et al. 2001, PNAS, Vol 98, No. 16, Conditional epidermal expression of TGFβ-1 blocks neonatal lethality but causes a reversible hyperplasia and alopecia). In addition, TGFβ-1 mutants have been associated with the delay of catagen onset in mice (*see* Foitzik, K et al, 2000). Finally, androgens that induce TGFβ-1 production in balding dermal papilla cells can inhibit epithelial cell growth (Inui, S, et al. 2002 FASEB J).

Summary of the invention

The current invention relates to peptides and supported peptides which bind TGFβ-1. Specifically, the invention is drawn to a cosmetic and/or pharmaceutical compound for modulating hair growth. The current invention discloses, among other things, peptides that bind and block binding of TGFβ-1, wherein the peptide is expressed in a protease-resistant scaffold. The scaffold may be a protease inhibitor, such as BBI, STI or Eglin chymotrypsin inhibitor.

In a first aspect, the invention is drawn to a cosmetic or pharmaceutical compound for modulating hair growth comprising a polypeptide or a peptide. In a preferred embodiment, the compound comprises a polypeptide.

In another preferred embodiment, the compound comprises a peptide. In a preferred embodiment, the peptide has an amino acid sequence selected from the group consisting of SEQ ID NO:1-SEQ ID NO:3 (as shown, respectively, in Table 1). In a preferred embodiment, the compound has a sequence, the sequence being at least 70%, preferably 80%, preferably 90%, preferably 95% homologous to the sequences set forth herein. In a preferred embodiment, the polypeptide has a molecular weight that is preferably between 500 Daltons and 30,000 Daltons, preferably between 1000 Daltons and 10,000 Daltons, preferably between 1500 Daltons to 8,000 Daltons.

In a preferred embodiment, modulation comprises treatment of at least one disease or condition that involves loss of hair. In a preferred embodiment, the disease or condition is at least one selected from the group consisting of inflammatory alopecias, pseudopelade, scleroderma, tick bites, lichen planus, psoriasis, lupus, seborrheic dermatitis, loose hair syndrome, hemochromatosis, alopecia androgenic, alopecia areata, cancer, conditions that affect defective hair fiber production and environmental factors that affect hair production. In a preferred embodiment, the disease is alopecia androgenic or alopecia areata.

In a preferred embodiment, the modulation comprises inhibition of hair growth (or removal and/or inhibition) for at least one disease or condition for which hair growth would not be desirable. In a preferred embodiment, the inhibition comprises depilation.

In a second aspect, the invention is drawn to a cosmetic or pharmaceutical compound for modulating hair growth comprising a peptide or a polypeptide and a scaffold, the peptide or polypeptide sequence being contained in the scaffold, preferably the peptide or polypeptide being a loop, preferably, the loop being closed by a disulfide bond. The peptide or polypeptide may be one that binds to TGFβ-1 and blocks its downstream activity. In a preferred embodiment, the scaffold is STI, Eglin or BBI. In a preferred embodiment, the preferred scaffold is BBI. In a preferred embodiment, the compound is a polypeptide.

In another preferred embodiment, the compound is a peptide. In a preferred embodiment, the peptide has an amino acid sequence selected from the group consisting of SEQ ID NO:1-SEQ ID NO:3 (as shown, respectively, in Table 1). In a preferred embodiment, the compound has a sequence, the sequence being at least 70%, preferably

80%, preferably 90%, preferably 95% homologous to the sequences set forth herein. In a preferred embodiment, the polypeptide has a molecular weight that is between 500 Daltons and 100,000 Daltons, preferably between 500 Daltons and 45,000 Daltons, preferably between 1000 Daltons and 12,000 Daltons, preferably from 1500 Daltons to 10,000 Daltons.

In a preferred embodiment, modulation comprises treatment of at least one disease or condition that involves loss of hair. In a preferred embodiment, the disease or condition is at least one selected from the group consisting of inflammatory alopecias, pseudopelade, scleroderma, tick bites, lichen planus, psoriasis, lupus, seborrheic dermatitis, loose hair syndrome, hemochromatosis, alopecia androgenic, alopecia areata, cancer, conditions that affect defective hair fiber production and environmental factors that affect hair production. In a preferred embodiment, the disease is alopecia androgenic or alopecia areata.

In a preferred embodiment, the modulation comprises inhibition of hair growth (or removal and/or inhibition) for at least one disease or condition for which hair growth would not be desirable. In a preferred embodiment, the inhibition comprises depilation.

In a third aspect, the invention is drawn to a cosmetic or pharmaceutical composition comprising a polypeptide or peptide, as set forth herein, and a physiologically acceptable carrier or excipient. Preferably, the compound is present in an amount of about 0.0001% to about 5% by weight based on the total weight of the composition. Also preferably, the compound is present in an amount of about 0.001% to about 0.5% by weight based on the total weight of the composition. The composition may be in the form of an emulsified vehicle, such as a nutrient cream or lotion, a stabilized gel or dispersion system, a treatment serum, a liposomal delivery system, a topical pack or mask, a surfactant-based cleansing system such as a shampoo or body wash, an aerosolized or sprayed dispersion or emulsion, a hair or skin conditioner, styling aid, or a pigmented product such as makeup.

Preferably, the carrier is at least one selected from the group consisting of water, propylene glycol, ethanol, propanol, glycerol, butylene glycol and polyethylene glycol.

In a fourth aspect, the invention is drawn to a method of decreasing TGFβ-1 activity, the method comprising applying to an organism in need thereof an effective amount of any one of the compounds set forth herein. The fourth aspect includes

applications drawn to hair treatment, as disclosed in the first three aspects, as well as other applications (e.g., wound healing, treatment of proliferative diseases, etc). In preferred embodiments, the fourth aspect includes compounds for treatment of an organism in need thereof.

Brief description of Figures

Figure 1A sets forth the plasmid map for pME30.16; Figure 1B sets forth the plasmid map for p2JM103-DNNDPI-BBI; Figure 1C sets forth the plasmid map for pCB04.

Figure 2 shows the amino acid sequence of BBI backbone used herein comprising approximately 71 amino acid residues. The loops are underlined.

Figure 3 shows the first screen data, BLA activity plotted against total BLA activity. The x-axis plots total BLA activity and the Y-axis plots bound BLA activity, as described in the Examples.

Detailed Description of the Preferred Embodiments

The invention will now be described in detail by way of reference only using the following definitions and examples. Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton, *et al.*, DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY, 2D ED., John Wiley and Sons, New York (1994), and Hale & Marham, THE HARPER COLLINS DICTIONARY OF BIOLOGY, Harper Perennial, NY (1991) provide one of skill with a general dictionary of many of the terms used in this invention. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. Practitioners are particularly directed to Sambrook *et al.*, 1989, and Ausubel FM *et al.*, 1993, for definitions and terms of the art. It is to be understood that this

invention is not limited to the particular methodology, protocols and reagents described, as these may vary.

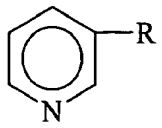
The headings provided herein are not limitations of the various aspects or embodiments of the invention which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification as a whole.

All publications cited herein are expressly incorporated herein by reference for the purpose of describing and disclosing compositions and methodologies which might be used in connection with the invention.

The term "organism" is used throughout the specification to describe an animal, preferably a human, to whom treatment, including prophylactic treatment, with the compounds according to the present invention is provided. For treatment of those infections, conditions or disease states which are specific for a specific animal such as a human patient, the term organism refers to that specific animal. In most instances, the term organism refers to a human patient.

The term "effective amount" is used throughout the specification to describe concentrations or amounts of compounds according to the present invention which may be used to produce a favorable change in the disease or condition treated, whether that change is hair growth or prevention of hair growth.

As used herein, "vitamin B_3 compound" means a compound having the formula:



wherein R is - $CONH_2$ (i.e., niacinamide), - COOH (i.e., nicotinic acid) or - CH_2OH (i.e., nicotinyl alcohol); derivatives thereof; and salts of any of the foregoing.

As used herein, "non-vasodilating" means that an ester does not commonly yield a visible flushing response after application to the skin in the subject compositions (the majority of the general population would not experience a visible flushing response, although such compounds may cause vasodilation not visible to the naked eye).

As used herein, "retinoid" includes all natural and/or synthetic analogs of Vitamin A or retinol-like compounds which possess the biological activity of Vitamin A in the skin as well as the geometric isomers and stereoisomers of these compounds.

As used herein, "silicone gum" means high molecular weight silicones having a weight average molecular weight in excess of about 200,000 and preferably from about 200,000 to about 4,000,000. Included are non-volatile polyalkyl and polyaryl siloxane gums.

As used herein, the term "polypeptide" refers to a compound made up of a single chain of amino acid residues linked by peptide bonds. The term "protein" herein may be synonymous with the term "polypeptide" or may refer, in addition, to a complex of two or more polypeptides.

As used herein, the term "expression" refers to the process by which a polypeptide is produced based on the nucleic acid sequence of the gene or the chemical synthetic peptide. The process includes both transcription and translation.

As used herein, the term "gene" means the segment of DNA involved in producing a polypeptide chain that may or may not include regions preceding or following the coding region.

As used herein, the term "nucleic acid molecule" includes RNA, DNA and cDNA molecules. It will be understood that as a result of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding a given protein, such as the mutant proteins of the invention, may be produced.

As used herein, the term "disulfide bridge" or "disulfide bond" refers to the bond formed between the sulphur atoms of cysteine residues in a polypeptide or a protein. In this invention, a disulfide bridge or disulfide bond may be non-naturally occurring and introduced by way of point mutation.

As used herein, the term "salt bridge" refers to the bond formed between oppositely charged residues, amino acids in a polypeptide or protein. In this invention, a salt bridge may be non-naturally occurring and introduced by way of point mutation.

As used herein, an "enzyme" refers to a protein or polypeptide that catalyzes a chemical reaction.

As used herein, the term "activity" refers to a biological activity associated with a particular protein, such as enzymatic activity associated with a protease. Another example

might be a protein binding to a receptor which causes measurable downstream effects, such as inhibition of the transition from anagen to catagen, as described herein. Biological activity refers to any activity that would normally be attributed to that protein by one skilled in the art.

As used herein, the term "protease" refers to an enzyme that degrades by hydrolyzing peptide bonds.

As used herein, "peptide bond" refers to the chemical bond between the carbonyl group of one amino acid and the amino group of another amino acid.

As used herein, "wild-type" refers to a sequence or a protein that is native or naturally occurring.

As used herein, "point mutations" refers to a change in a single nucleotide of DNA, especially where that change will result in a sequence change in a protein.

As used herein, "mutant" refers to a version of an organism or protein where the version is other than wild-type. The change may be affected by methods well known to one skilled in the art, for example, by point mutation in which the resulting protein may be referred to as a mutant.

As used herein, "mutagenesis" refers to the process of affecting a change from a wild-type into a mutant or variant.

As used herein, "substituted" and "modified" are used interchangeably and refer to a sequence, such as an amino acid sequence comprising a polypeptide, that includes a deletion, insertion, replacement or interruption of a naturally occurring sequence. Often in the context of the invention, a substituted sequence shall refer, for example, to the replacement of a naturally occurring residue.

As used herein, "loop" refers to a sequence of amino acids, for example 3-20 amino acids, preferably 5-15 amino acids, preferably 5-10 amino acids, preferably 7-9 amino acids, which connects structural elements of a protein. Such elements may be, for example, beta sheets and helical elements and the connecting loop of a beta-hairpin. The loop may be further stabilized through the use of covalent linkages, specifically by the disulfide bonds, especially as provided herein. Or, alternatively, for example, the loops may be stabilized by the use of, for example, amides, hydrogen bonds or salt bridges. These loops are typically in the surface of proteins and may be altered, as provided herein, for example, to confer additional properties on the requisite proteins.

As used herein, "oligonucleotides" refers to a short nucleotide sequence which may be used, for example, as a primer in a reaction used to create mutant proteins.

As used herein, "codon" refers to a sequence of three nucleotides in a DNA or mRNA molecule that represents the instruction for incorporation of a specific amino acid into a polypeptide chain.

As used herein, "anagen" refers to the active growth phase of hair follicles. In the anagen phase, cells in the root of the hair are dividing rapidly, adding to the hair shaft. During this phase the hair grows about 1 cm every 28 days. Scalp hair stays in this active phase of growth for 2-6 years.

As used herein, "catagen" refers to the hair growth phase that occurs at the end of the anagen phase. It signals the end of the active growth of a hair. This phase lasts for about 2-3 weeks while a club hair is formed.

As used herein, "telogen" refers to the resting phase of the hair follicle. At any given time, 10%-15% of all hairs are in the telogen phase. This phase lasts for about 100 days for hairs on the scalp and much longer for hairs on the eyebrow, eyelash, arm and leg. During this phase the hair follicle is completely at rest and the club hair is completely formed. Pulling out a hair in this phase will reveal a solid, hard, dry, white material at the root. About 25-100 telogen hairs are shed normally each day.

As used herein, "depilation" refers to the act of removing hair.

As used herein, "alopecia" refers to loss of hair. Hair loss is thought to proceed by mechanisms involving TGFβ-1, as disclosed herein.

The current invention relates to peptides and supported peptides which bind TGFβ-1. Specifically, the invention is drawn to a cosmetic and/or pharmaceutical compound for modulating hair growth. The current invention discloses, among other things, peptides that bind and block binding of TGFβ-1, wherein the peptide is expressed in a protease-resistant scaffold. The scaffold may be a protease inhibitor, such as BBI, STI or Eglin chymotrypsin inhibitor.

In a first aspect, the invention is drawn to a cosmetic or pharmaceutical compound for modulating hair growth comprising a polypeptide or a peptide. In a preferred embodiment, the compound comprises a polypeptide.

In another preferred embodiment, the compound comprises a peptide. In a preferred embodiment, the peptide has an amino acid sequence selected from the group consisting of SEQ ID NO:1-SEQ ID NO:3 (as shown, respectively, in Table 1). In a preferred embodiment, the compound has a sequence, the sequence being at least 70%, preferably 80%, preferably 90%, preferably 95% homologous to the sequences set forth herein. In a preferred embodiment, the polypeptide has a molecular weight that is preferably between 500 Daltons and 30,000 Daltons, preferably between 1000 Daltons and 10,000 Daltons, preferably between 1500 Daltons to 8,000 Daltons.

In a preferred embodiment, modulation comprises treatment of at least one disease or condition that involves loss of hair. In a preferred embodiment, the disease or condition is at least one selected from the group consisting of inflammatory alopecias, pseudopelade, scleroderma, tick bites, lichen planus, psoriasis, lupus, seborrheic dermatitis, loose hair syndrome, hemochromatosis, alopecia androgenic, alopecia areata, cancer, conditions that affect defective hair fiber production and environmental factors that affect hair production. In a preferred embodiment, the disease is alopecia androgenic or alopecia areata.

In a preferred embodiment, the modulation comprises inhibition of hair growth (or removal and/or inhibition) for at least one disease or condition for which hair growth would not be desirable. In a preferred embodiment, the inhibition comprises depilation.

In a second aspect, the invention is drawn to a cosmetic or pharmaceutical compound for modulating hair growth comprising a peptide or a polypeptide and a scaffold, the peptide or polypeptide sequence being contained in the scaffold, preferably the peptide

or polypeptide being a loop, preferably, the loop being closed by a disulfide bond. The peptide or polypeptide may be one that binds to TGFβ-1 and blocks its downstream activity. In a preferred embodiment, the scaffold is STI, Eglin or BBI. In a preferred embodiment, the preferred scaffold is BBI. In a preferred embodiment, the compound is a polypeptide.

In another preferred embodiment, the compound is a peptide. In a preferred embodiment, the peptide has an amino acid sequence selected from the group consisting of SEQ ID NO:1-SEQ ID NO:3 (as set forth, respectively, in Table 1). In a preferred embodiment, the compound has a sequence, the sequence being at least 70%, preferably 80%, preferably 90%, preferably 95% homologous to the sequences set forth herein. In a preferred embodiment, the polypeptide has a molecular weight that is between 500 Daltons and 100,000 Daltons, preferably between 500 Daltons and 45,000 Daltons, preferably between 1000 Daltons and 12,000 Daltons, preferably from 1500 Daltons to 10,000 Daltons.

In a preferred embodiment, modulation comprises treatment of at least one disease or condition that involves loss of hair. In a preferred embodiment, the disease or condition is at least one selected from the group consisting of inflammatory alopecias, pseudopelade, scleroderma, tick bites, lichen planus, psoriasis, lupus, seborrheic dermatitis, loose hair syndrome, hemochromatosis, alopecia androgenic, alopecia areata, cancer, conditions that affect defective hair fiber production and environmental factors that affect hair production. In a preferred embodiment, the disease is alopecia androgenic or alopecia areata.

In a preferred embodiment, the modulation comprises inhibition of hair growth (or removal and/or inhibition) for at least one disease or condition for which hair growth would not be desirable. In a preferred embodiment, the inhibition comprises depilation.

The current invention is directed to a peptide or a polypeptide, a loop and a protease-resistant scaffold. Flexible native loops are found on the surface of most protein modules and exist as short stretches of amino acids that connect regions of defined secondary structure. Although crystallographic and NMR studies show that native loops are usually less well defined than alpha-helices and beta-sheets, their conformational freedom is normally restricted substantially compared with free peptides. Consequently, the binding

activities of native loops in proteins usually differ significantly from those of the corresponding linear amino acid sequence .

The loops of the current invention bind proteins, such as TGFβ-1. Binding the loop to the protein prevents the protein from binding to its target. Thus, binding interactions are thought to be disrupted by binding the loop to the protein. As a result, bioactivity can be altered.

The current invention provides scaffolds to stabilize the loops. STI, BBI and EglinC have native loops that bind to and inhibit proteases. STI and BBI native loops may be replaced with the polypeptides and/or peptides of the invention, the loops. As disclosed herein, the sequences may be replaced with inhibitors or enhancers of TGFβ-1. Additionally, STI and BBI native loops may be replaced with sequences that have been isolated with techniques such as phage display as, for example, the TGFβ-1 binding proteins disclosed herein.

A native loop may be replaced with a loop which is, for example, 3 to 20 amino acids in length, preferably 5 to 15 amino acids in length, preferably 5 to 10 amino acids in length. Longer sequences may be used as long as they provide binding and/or inhibition. In addition, peptides suitable for use as replacements of the native loop(s) can form constrained loops, i.e., a loop formed by the presence to a disulfide bond between two cysteine residues. In specific embodiments, the peptides are between 7 and 9 amino acids in length.

There are several advantages to using scaffolds to stabilize peptide sequences. First, biological activity of the peptide may be expected to be higher (or may be expected to modulate biological function) as a result of limiting peptide flexibility and reducing the entropic cost of fixing the polypeptide sequence in the bioactive conformation. Second, structural information can be obtained by x-ray crystallography to guide affinity maturation. Third, the sequence presented on a structural scaffold may be more resistant to proteolytic degradation in different biological applications. Finally, the chimeric construction can be obtained in large amount in low cost biological expression systems for industrial applications (*see*, for example, Attorney Docket Numbers GC815P and GC817P both of which are incorporated by reference herein and to which this application claims priority).

Compounds of the present invention bind TGFβ-1 (*see,* Examples). Binding can, for example, absorb extracellular TGFβ-1 and, thus, prevent TGFβ-1 from interacting with its cognate ligand, thereby preventing a downstream biological effect. In the present case, binding prevents TGFβ-1 from interacting with its cognate receptor and inhibits transition from the anagen to the catagen promoting hair growth and preventing hair loss.

BBI represents a class of protein scaffolds whose binding to proteases is mediated by an exposed native loop that is fixed in a characteristic canonical conformation and which fits into the active site in a manner thought to be similar to that of a substrate (Laskowski & Kato, 1980; Bode & Huber, 1992). The native loop is frequently constrained by the presence of disulfide bridges and/or extensive hydrogen-bonding networks that act to lock the structure into the correct canonical structure. The sequence of this loop determines the specificity of the inhibition, which mirrors the specificity of proteinases for their substrates. For example, most trypsin inhibitors have Arg or Lys as their P1 residue. Inhibitors of the BBI family have a network of conserved disulfide bridges that help form a symmetrical structure of two tricyclic domains (Chen *et al.*, 1992; Werner & Wemmer, 1992; Lin *et al.*, 1993), each containing an independent serine proteinase binding site. The native binding loop is contained within a region joined by disulfide bridges formed between cysteine residues. The identity of the amino acid residue at the P1 site on each domain is the main determinant of the serine proteinase inhibited. Native domains possess lysine or arginine for trypsin, leucine or tyrosine for chymotrypsin and alanine for elastase (Ikenaka & Norioka, 1986). In addition, serine is highly conserved at the P'1 position and proline at the P'3 position. The individual native loop regions of BBI are well suited for protein loop grafting of previously identified cysteine constrained peptides that bind to targets selectively, as disclosed herein.

Numerous isoforms of BBI have been characterized; SEQ ID NO: 4 (*see*, Figure 2) shows the amino acid sequence of BBI backbone used herein comprising approximately 71 amino acid residues. In addition, BBI may be truncated with as many as 10 amino acid residues being removed from either the N- or C- terminal. Any of the isoforms disclosed herein, as well as those known in the art, may be used as a scaffold.

The disclosed invention has several advantages over creation of, for example, chimeric proteins. Transfer of an entire protein can be difficult when, for example, a

protein domain of interest carries more than one important biological activity. Maintaining one activity (*e.g.* functionally significant domain-domain interactions) while altering another (*e.g.* high affinity binding to a co-factor or receptor) can be problematic. The invention, as disclosed herein, overcomes that limitation, as loops are transferred, instead of entire domains.

The compounds of the invention may comprise one or more mutations in addition to those set out above. Other mutations, such as deletions, insertions, substitutions, transversions, transitions and inversions, at one or more other locations, may also be included.

The compound may also comprise a conservative substitution that may occur as a like-for-like substitution (e.g., basic for basic, acidic for acidic, polar for polar etc.) Non-conservative substitutions may also occur, i.e. from one class of residue to another or alternatively involving the inclusion of unnatural amino acids such as ornithine, diaminobutyric acid ornithine, norleucine ornithine, pyriylalanine, thienylalanine, naphthylalanine and phenylglycine.

The sequences may also have deletions, insertions or substitutions of amino acid residues that produce a silent change and result in a functionally equivalent substance. Deliberate amino acid substitutions may be made on the basis of similarity in amino acid properties (such as polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues) and it is therefore useful to group amino acids together in functional groups. Amino acids can be grouped together based on the properties of their side chain alone. However it is more useful to include mutation data as well. The sets of amino acids thus derived are likely to be conserved for structural reasons. These sets can be described in the form of a Venn diagram (Livingstone C.D. and Barton G.J. (1993) "Protein sequence alignments: a strategy for the hierarchical analysis of residue conservation" *Comput.Appl Biosci.* 9: 745-756)(Taylor W.R. (1986) "The classification of amino acid conservation" *J.Theor.Biol.* 119; 205-218). Conservative substitutions may be made, for example according to the table below that describes a generally accepted Venn diagram grouping of amino acids.

Set		Sub-set	
Hydrophobic	F W Y H K M I L V A G C	Aromatic	F W Y H
		Aliphatic	I L V
Polar	W Y H K R E D C S T N Q	Charged	H K R E D
		Positively charged	H K R
		Negatively charged	E D
Small	V C A G S P T N D	Tiny	A G S

Variant amino acid sequences may also include suitable spacer groups inserted between any two amino acid residues of the sequence including alkyl groups such as methyl, ethyl or propyl groups in addition to amino acid spacers such as glycine or β-alanine residues. A further form of variation involves the presence of one or more amino acid residues in peptoid form.

Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. Available computer programs can calculate % homology between two or more sequences. % homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without

penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible - reflecting higher relatedness between the two compared sequences - will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (Devereux et al 1984 Nuc. Acids Research 12 p387). Examples of other software than can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel et al., 1999 Short Protocols in Molecular Biology, 4th Ed – Chapter 18), FASTA (Altschul et al., 1990 J. Mol. Biol. 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel et al., 1999, Short Protocols in Molecular Biology, pages 7-58 to 7-60). However, for some applications, it is preferred to use the GCG Bestfit program. BLAST 2 Sequences is also available for comparing protein and nucleotide sequence (see FEMS Microbiol Lett 1999 174(2): 247-50; FEMS Microbiol Lett 1999 177(1): 187-8 and tatiana@ncbi.nlm.nih.gov).

Although the final % homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a

custom symbol comparison table if supplied (see user manual for further details). For some applications, it is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

Alternatively, percentage homologies may be calculated using the multiple alignment feature in DNASIS™ (Hitachi Software), based on an algorithm, analogous to CLUSTAL (Higgins DG & Sharp PM (1988), Gene 73(1), 237-244).

Once the software has produced an optimal alignment, it is possible to calculate % homology, preferably % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

Embodiments of the first and second aspects of the invention, as disclosed above, provide a nucleic acid encoding any of the compounds, as set forth above, as well as complements thereof. In another preferred embodiment, the invention provides vectors comprising a compound, as disclosed herein, cells comprising the compound and methods of expressing the compound.

One skilled in the art will be aware of the relationship between nucleic acid sequence and polypeptide sequence, in particular, the genetic code and the degeneracy of this code, and will be able to construct such nucleic acids without difficulty. For example, one skilled in the art will be aware that for each amino acid substitution in the compound sequence there may be one or more codons that encode the substitute amino acid. Accordingly, it will be evident that, depending on the degeneracy of the genetic code with respect to that particular amino acid residue, one or more nucleic acid sequences may be generated corresponding to that compound polypeptide sequence.

Mutations in amino acid sequence and nucleic acid sequence may be made by any of a number of techniques, as known in the art. In particularly preferred embodiments, the mutations are introduced into parent sequences by means of PCR (polymerase chain reaction) using appropriate primers. The parent enzymes may be modified at the amino acid level or the nucleic acid level to generate the compound sequences described herein. Therefore, a preferred embodiment provides for the generation of compounds by introducing one or more corresponding codon changes in the nucleotide sequence encoding a compound.

It will be appreciated that the above codon changes can be made in any compound nucleic acid sequence. For example, sequence changes can be made to any of the homologous sequences described herein.

The compound may comprise the "complete" protein, i.e., in its entire length as it occurs in nature (or as mutated) or it may comprise a truncated form thereof. The compound may accordingly be so truncated or be "full-length". The truncation may be at the N-terminal end or the C-terminal end. The compound may lack one or more portions, such as sub-sequences, signal sequences, domains or moieties, whether active or not.

In a yet further alternative, the nucleotide sequence encoding the compound may be prepared synthetically by established standard methods, e.g. the phosphoroamidite method described by Beucage S.L. *et al.,* (1981) *Tetrahedron Letters* 22, p 1859-1869 or the method described by Matthes *et al.,* (1984) *EMBO J.* 3, p 801-805. In the phosphoroamidite method, oligonucleotides are synthesised, e.g. in an automatic DNA synthesiser, purified, annealed, ligated and cloned in appropriate vectors.

The nucleotide sequence may be of mixed genomic and synthetic origin, mixed synthetic and cDNA origin or mixed genomic and cDNA origin, prepared by ligating fragments of synthetic, genomic or cDNA origin in accordance with standard techniques. Each ligated fragment corresponds to various parts of the entire nucleotide sequence. The DNA sequence may also be prepared by polymerase chain reaction (PCR) using specific primers, for instance as described in US 4,683,202 or in Saiki R K *et al.,* (*Science* (1988) 239, pp 487-491).

The nucleotide sequences described here, and suitable for use in the methods and compositions described here may include within them synthetic or modified nucleotides. A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones and/or the addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. For the purposes of this document, it is to be understood that the nucleotide sequences described herein may be modified by any method available in the art. Such modifications may be carried out in order to enhance the *in vivo* activity or life span of nucleotide sequences.

A preferred embodiment of the invention provides for nucleotide sequences and the use of nucleotide sequences that are complementary to the sequences presented herein, or any derivative, fragment or derivative thereof.

The polynucleotides may be used to produce a primer, e.g. a PCR primer, a primer for an alternative amplification reaction, a probe e.g. labelled with a revealing label by conventional means using radioactive or non-radioactive labels or the polynucleotides may be cloned into vectors. Such primers, probes and other fragments will be at least 15, preferably at least 20, for example at least 25, 30 or 40 nucleotides in length, and are also encompassed by the term polynucleotides.

Polynucleotides such as DNA polynucleotides and probes may be produced recombinantly, synthetically or by any means available to those of skill in the art. They may also be cloned by standard techniques. In general, primers will be produced by synthetic means, involving a stepwise manufacture of the desired nucleic acid sequence one nucleotide at a time. Techniques for accomplishing this using automated techniques are readily available in the art.

Longer polynucleotides will generally be produced using recombinant means, for example using PCR (polymerase chain reaction) cloning techniques. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable cloning vector. Preferably, the variant sequences are at least as biologically active as the sequences presented herein.

A preferred embodiment of the invention includes sequences that are complementary to the compound or sequences that are capable of hybridising either to the nucleotide sequences of the compounds (including complementary sequences of those presented herein), as well as nucleotide sequences that are complementary to sequences that can hybridise to the nucleotide sequences of the compounds (including complementary sequences of those presented herein). A preferred embodiment provides polynucleotide sequences that are capable of hybridising to the nucleotide sequences presented herein under conditions of intermediate to maximal stringency.

A preferred embodiment includes nucleotide sequences that can hybridise to the nucleotide sequence of the compound nucleic acid, or the complement thereof, under stringent conditions (e.g. 50°C and 0.2xSSC). More preferably, the nucleotide sequences

can hybridise to the nucleotide sequence of the compound, or the complement thereof, under high stringent conditions (e.g. 65°C and 0.1xSSC).

It may be desirable to mutate the sequence in order to prepare a compound. Accordingly, a mutant may be prepared from the compounds provided herein. Mutations may be introduced using synthetic oligonucleotides. These oligonucleotides contain nucleotide sequences flanking the desired mutation sites. A suitable method is disclosed in Morinaga *et al.*, (*Biotechnology* (1984) 2, p646-649). Another method of introducing mutations into enzyme-encoding nucleotide sequences is described in Nelson and Long (*Analytical Biochemistry* (1989), 180, p 147-151). A further method is described in Sarkar and Sommer (*Biotechniques* (1990), 8, p404-407 – "The megaprimer method of site directed mutagenesis"). Other methods to mutate the sequence are employed and disclosed herein.

In a preferred embodiment, the sequence for use in the methods and compositions described here is a recombinant sequence – i.e. a sequence that has been prepared using recombinant DNA techniques. Such techniques are explained, for example, in the literature, for example, J. Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press.

Another embodiment provides vectors comprising the compound, cells comprising the compound and methods of expressing the compound. The nucleotide sequence for use in the methods and compositions described herein may be incorporated into a recombinant replicable vector. The vector may be used to replicate and express the nucleotide sequence, in enzyme form, in and/or from a compatible host cell. Expression may be controlled using control sequences, e.g., regulatory sequences. The enzyme produced by a host recombinant cell by expression of the nucleotide sequence may be secreted or may be contained intracellularly depending on the sequence and/or the vector used. The coding sequences may be designed with signal sequences which direct secretion of the substance coding sequences through a particular prokaryotic or eukaryotic cell membrane. Polynucleotides can be incorporated into a recombinant replicable vector. The vector may be used to replicate the nucleic acid in a compatible host cell. The vector comprising the

polynucleotide sequence may be transformed into a suitable host cell. Suitable hosts may include bacterial, yeast, insect, fungal cells and mammalian cells.

Compounds and their polynucleotides may be expressed by introducing a polynucleotide into a replicable vector, introducing the vector into a compatible host cell and growing the host cell under conditions which bring about replication of the vector. The vector may be recovered from the host cell.

The compound nucleic acid may be operatively linked to transcriptional and translational regulatory elements active in a host cell of interest. The compound nucleic acid may also encode a fusion protein comprising signal sequences such as, for example, those derived from the glucoamylase gene from *Schwanniomyces occidentalis,* α-factor mating type gene from *Saccharomyces cerevisiae* and the TAKA-amylase from *Aspergillus oryzae*. Alternatively, the compound nucleic acid may encode a fusion protein comprising a membrane binding domain.

The compound may be expressed at the desired levels in a host organism using an expression vector. An expression vector comprising a compound nucleic acid can be any vector capable of expressing the gene encoding the compound nucleic acid in the selected host organism, and the choice of vector will depend on the host cell into which it is to be introduced. Thus, the vector can be an autonomously replicating vector, i.e. a vector that exists as an episomal entity, the replication of which is independent of chromosomal replication, such as, for example, a plasmid, a bacteriophage or an episomal element, a minichromosome or an artificial chromosome. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome.

The expression vector typically includes the components of a cloning vector, such as, for example, an element that permits autonomous replication of the vector in the selected host organism and one or more phenotypically detectable markers for selection purposes. The expression vector normally comprises control nucleotide sequences encoding a promoter, operator, ribosome binding site, translation initiation signal and optionally, a repressor gene or one or more activator genes. Additionally, the expression vector may comprise a sequence coding for an amino acid sequence capable of targeting the compound to a host cell organelle such as a peroxisome or to a particular host cell

compartment. Such a targeting sequence includes but is not limited to the sequence SKL. For expression under the direction of control sequences, the nucleic acid sequence the compound is operably linked to the control sequences in proper manner with respect to expression.

Preferably, a polynucleotide in a vector is operably linked to a control sequence that is capable of providing for the expression of the coding sequence by the host cell, i.e. the vector is an expression vector. The control sequences may be modified, for example, by the addition of further transcriptional regulatory elements to make the level of transcription directed by the control sequences more responsive to transcriptional modulators. The control sequences may in particular comprise promoters.

In the vector, the nucleic acid sequence encoding for the compound is operably combined with a suitable promoter sequence. The promoter can be any DNA sequence having transcription activity in the host organism of choice and can be derived from genes that are homologous or heterologous to the host organism. Examples of suitable promoters for directing the transcription of the modified nucleotide sequence, such as compound nucleic acids, in a bacterial host include the promoter of the *lac* operon of *E. coli,* the *Streptomyces coelicolor* agarase gene *dagA* promoters, the promoters of the *Bacillus licheniformis* α-amylase gene *(amyL),* the aprE promoter of *Bacillus subtilis,* the promoters of the *Bacillus stearothermophilus* maltogenic amylase gene *(amyM),* the promoters of the *Bacillus amyloliquefaciens* α-amylase gene *(amyQ),* the promoters of the *Bacillus subtilis xylA and xylB* genes and a promoter derived from a *Lactococcus* sp.-derived promoter including the P170 promoter. When the gene encoding the compound is expressed in a bacterial species such as *E. coli,* a suitable promoter can be selected, for example, from a bacteriophage promoter including a T7 promoter and a phage lambda promoter. For transcription in a fungal species, examples of useful promoters are those derived from the genes encoding the *Aspergillus oryzae* TAKA amylase, *Rhizomucor miehei* aspartic proteinase, *Aspergillus niger* neutral α-amylase, *A. niger* acid stable α-amylase, *A. niger glucoamylase, Rhizomucor miehei lipase, Aspergillus oryzae* alkaline protease, *Aspergillus oryzae* triose phosphate isomerase or *Aspergillus nidulans* acetamidase. Examples of suitable promoters for the expression in a yeast species include but are not limited to the

Gal 1 and Gal 10 promoters of *Saccharomyces cerevisiae* and the *Pichia pastoris AOX1 or* AOX2 promoters.

Examples of suitable bacterial host organisms are gram positive bacterial species such *as Bacillaceae including Bacillus subtilis, Bacillus licheniformis, Bacillus lentus, Bacillus brevis, Bacillus stearothermophilus, Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus lautus, Bacillus megaterium and Bacillus thuringiensis, Streptomyces* species such as *Streptomyces murinus,* lactic acid bacterial species in*cluding Lactococcus* spp. such as *Lactococcus lactis, Lactobacillus* spp. including *Lactobacillus reuteri, Leuconostoc spp., Pediococcus* spp. and *Streptococcus spp.* Alternatively, strains of a gram-negative bacterial species belonging to *Enterobacteria*ceae including *E. coli,* or to *Pseudomonadaceae* can be selected as the host organism.

A suitable yeast host organism can be selected from the biotechnologically relevant yeasts species such as but not limited to yeast species such as *Pichia sp., Hansenula sp or Kluyveromyces, Yarrowinia* species or a species of *Saccharomyces including Saccharomyces cerevisiae* or a species belonging to *Schizosaccharomyce* such as, for example, *S. Pombe* species. Preferably a strain of the methylotrophic yeast species *Pichia pastoris* is used as the host organism. Preferably the host organism is a *Hansenula* species. Suitable host organisms among filamentous fungi include species of *Aspergillus, e.g. Aspergillus niger, Aspergillus oryzae, Aspergillus tubigensis, Aspergillus awamori or Aspergillus nidulans.* Alternatively, strains of a *Fusarium* species, e.g. *Fusarium oxysporum* or of a *Rhizomucor* species such as *Rhizomucor miehei* can be used as the host organism. Other suitable strains include *Thermomyces* and *Mucor* species.

Host cells comprising polynucleotides may be used to express polypeptides, such as the compounds disclosed herein, fragments, homologues, variants or derivatives thereof. Host cells may be cultured under suitable conditions which allow expression of the proteins. Expression of the polypeptides may be constitutive such that they are continually produced, or inducible, requiring a stimulus to initiate expression. In the case of inducible expression, protein production can be initiated when required by, for example, addition of an inducer substance to the culture medium, for example dexamethasone or IPTG. Polypeptides can be extracted from host cells by a variety of techniques known in the art, including enzymatic, chemical and/or osmotic lysis and physical disruption. Polypeptides

may also be produced recombinantly in an *in vitro* cell-free system, such as the TnT™ (Promega) rabbit reticulocyte system (*Roche in vitro systems*).

In a third aspect, the invention is drawn to a cosmetic or pharmaceutical composition comprising a polypeptide or peptide, as set forth herein, and a physiologically acceptable carrier or excipient. Preferably, the compound is present in an amount of about 0.0001% to about 5% by weight based on the total weight of the composition. Also preferably, the compound is present in an amount of about 0.001% to about 0.5% by weight based on the total weight of the composition. The composition may be in the form of an emulsified vehicle, such as a nutrient cream or lotion, a stabilized gel or dispersion system, a treatment serum, a liposomal delivery system, a topical pack or mask, a surfactant-based cleansing system such as a shampoo or body wash, an aerosolized or sprayed dispersion or emulsion, a hair or skin conditioner, styling aid, or a pigmented product such as makeup.

Preferably, the carrier is at least one selected from the group consisting of water, propylene glycol, ethanol, propanol, glycerol, butylene glycol and polyethylene glycol.

Liposomes may comprises, at least, water and one or more ingredients capable of forming lipid bilayer vesicles that can hold one or more functional or active ingredient(s). Non-limiting examples of ingredients capable of forming lipid bilayer vesicles include: phospholipids, hydrogenated phosphatidylcholine, lecithin, cholesterol and sphingolipids. Non-limiting examples of functional or active ingredients that can be delivered from liposomes include: vitamins and their derivatives, antioxidants, proteins and peptides, keratolytic agents, bioflavinoids, terpenoids, phytochemicals, and extracts of plant, marine or fermented origin. In a preferred embodiment, the composition further comprises a skin care or hair care active. Active ingredients can include any of a wide variety of ingredients such as are known in the art. (See e.g., McCutcheon's Functional Materials, North American and International Editioins, (2003), published by MC Publishing Co.) Preferably, such actives include but are not limited to antioxidants, such as tocopheryl and ascorbyl derivatives, bioflavinoids, terpenoids, synthetics and the like, vitamins and vitamin derivatives, hydroxyl- and polyhydroxy acids and their derivatives, such as AHAs and BHAs and their reaction products, peptides and polypeptides and their derivatives, such as glycopeptides and lipophilized peptides, heat shock proteins and cytokines, enzymes and enzymes inhibitors and their derivatives, such as proteases, MMP inhibitors, catalases,

glucose oxydase and superoxide dismutase, amino acids and their derivatives, bacterial, fungal and yeast fermentation products and their derivatives, including mushrooms, algae and seaweed and their derivatives, phytosterols and plant and plant part extracts and their derivatives and phospholipids and their derivatives, anti-dandruff agents such as zinc pyrithione and and delivery systems containing them, as provided herein.

In a preferred embodiment, the skin care active is selected from the group consisting of a Vitamin B3 component, panthenol, Vitamin E, Vitamin E acetate, retinol, retinyl propionate, retinyl palmitate, retinoic acid, Vitamin C, theobromine, alpha-hydroxyacid, farnesol, phytrantriol, salicylic acid, palmityl peptapeptide-3 and mixtures thereof. In a preferred embodiment, the Vitamin B3 component is niacinamide. The compositions herein may comprise a skin care active at a level from about 0.0001% to about 20%, preferably from about 0.001% to about 5%, more preferably from about 0.01% to about 2%, by weight.

Exemplary derivatives of the foregoing vitamin B_3 compounds include nicotinic acid esters, including non-vasodilating esters of nicotinic acid, nicotinyl amino acids, nicotinyl alcohol esters of carboxylic acids, nicotinic acid N-oxide and niacinamide N-oxide.

Suitable esters of nicotinic acid include nicotinic acid esters of C_1-C_{22}, preferably C_1-C_{16}, more preferably C_1-C_6 alcohols. The alcohols are suitably straight-chain or branched chain, cyclic or acyclic, saturated or unsaturated (including aromatic), and substituted or unsubstituted. The esters are preferably non-vasodilating.

Non-vasodilating esters of nicotinic acid include tocopherol nicotinate and inositol hexanicotinate; tocopherol nicotinate is preferred. A more complete description of vitamin B_3 compounds is given in WO 98/22085. Preferred vitamin B_3 compounds are niacinamide and tocopherol nicotinate.

Another suitable skin care active is a retinoid. The retinoid is preferably retinol, retinol esters (e.g., C_2 - C_{22} alkyl esters of retinol, including retinyl palmitate, retinyl acetate, retinyl proprionate), retinal, and/or retinoic acid (including all-trans retinoic acid and/or 13-cis-retinoic acid), more preferably retinoids other than retinoic acid. These compounds are well known in the art and are commercially available from a number of

sources, e.g., Sigma Chemical Company, and Boehringer Mannheim. Preferred retinoids are retinol, retinyl palmitate, retinyl acetate, retinyl proprionate, retinal, retinoic acid and combinations thereof. More preferred are retinol, retinoic propionate, retinoic acid and retinyl palmitate. The retinoid may be included as the substantially pure material, or as an extract obtained by suitable physical and/or chemical isolation from natural (e.g., plant) sources. When a retinoid is included in the compositions herein, it will preferably comprise from about 0.005% to about 2%, preferably from about 0.01% to about 1% retinoid. Retinol is preferably used in an amount of from about 0.01% to about 0.15%; retinol esters are preferably used in an amount of from about 0.01% to about 2% (e.g., about 1%).

The compositions herein can comprise a safe and effective amount of a dermatologically acceptable carrier, suitable for topical application to the skin or hair within which the essential materials and optional other materials are incorporated to enable the essential materials and optional components to be delivered to the skin or hair at an appropriate concentration. The carrier can thus act as a diluent, dispersant, solvent or the like for the essential components which ensures that they can be applied to and distributed evenly over the selected target at an appropriate concentration.

An effective amount of one or more compounds described herein may also be included in compositions to be applied to keratinous materials such as nails and hair, including but not limited to those useful as hair spray compositions, hair styling compositions, hair shampooing and/or conditioning compositions, compositions applied for the purpose of hair growth regulation and compositions applied to the hair and scalp for the purpose of treating seborrhoea, dermatitis and/or dandruff.

An effective amount of one or more compounds described herein may be included in compositions suitable for topical application to the skin or hair. These compositions can be in the form of creams, lotions, gels, suspensions dispersions, microemulsions, nanodispersions, microspheres, hydrogels, emulsions (e.g., oil-in-water and water-in-oil, as well as multiple emulsions) and multilaminar gels and the like (*see*, for example, The Chemistry and Manufacture of Cosmetics, Schlossman et al., 1998, incorporated by reference, herein), and may be formulated as aqueous or silicone compositions or may be formulated as emulsions of one or more oil phases in an aqueous continuous phase (or an aqueous phase in an oil phase).

The type of carrier utilized in the present invention depends on the type of product form desired for the composition. The carrier can be solid, semi-solid or liquid. Suitable carriers are liquid or semi-solid, such as creams, lotions, gels, sticks, ointments, pastes, sprays and mousses. Preferably the carrier is in the form of a lotion, cream or a gel, more preferably one which has a sufficient thickness or yield point to prevent the particles from sedimenting. The carrier can itself be inert or it can possess dermatological benefits of its own. The carrier may be applied directly to the skin and/or hair or it may be applied via a woven or non-woven wipe or cloth. It may also be in the form of a patch, mask or wrap. It may also be aerosolized or otherwise sprayed or pumped onto the skin and/or hair. The carrier should also be physically and chemically compatible with the essential components described herein, and should not unduly impair stability, efficacy or other use benefits associated with the compositions of the present invention.

Preferred carriers contain a dermatologically acceptable, hydrophilic diluent. Suitable hydrophilic diluents include water, organic hydrophilic diluents such as $C_2 - C_{10}$, preferably $C_2 - C_6$, preferably, $C_3 - C_6$ monohydric alcohols and low molecular weight glycols and polyols, including propylene glycol, polyethylene glycol polypropylene glycol, glycerol, butylene glycol, 1,2,4-butanetriol, sorbitol esters, 1,2,6-hexametriol, pentylene glycol, hexylene glycol, sorbitol esters, ethoxylated ethers, propoxylated ethers and combinations thereof. The diluent is preferably liquid. Water is a preferred diluent. The composition preferably comprises at least about 20% of the hydrophilic diluent.

Suitable carriers may also comprise an emulsion comprising a hydrophilic phase, especially an aqueous phase, and a hydrophobic phase e.g., a lipid, oil or oily material. As well known to one skilled in the art, the hydrophilic phase will be dispersed in the hydrophobic phase, or vice versa, to form respectively hydrophilic or hydrophobic dispersed and continuous phases, depending on the composition of ingredients. In emulsion technology, the term "dispersed phase" is a term well-known to one skilled in the art which means that the phase exists as small particles or droplets that are suspended in and surrounded by a continuous phase. The dispersed phase is also known as the internal or discontinuous phase. The emulsion may be or comprise (e.g., in a triple or other multi-phase emulsion) an oil-in-water emulsion or a water-in-oil emulsion such as a water-in-silicone emulsion. Oil-in-water emulsions typically (TOM: preferably?) comprise from

about 1% to about 60% (preferably about 1% to about 30%) of the dispersed hydrophobic phase and from about 1% to about 99% (preferably from about 10% to about 90%) of the continuous hydrophilic phase; water-in-oil emulsions typically comprise from about 1% to about 98% (preferably from about 40% to about 90%) of the dispersed hydrophilic phase and from about 1% to about 50% (preferably about 1% to about 30%) of the continuous hydrophobic phase.

The carrier might also include one or more components that facilitate penetration through the upper stratum corneum barrier to the lower levels of the skin. Examples of penetration enhancers include, but are not limited to, propylene glycol, azone, ethoxydiglycol, dimethyl isosorbide, urea, ethanol and dimethyl sulfoxide. Other examples include, but are not limited to, microemulsions, liposomes and nanoemulsions.

The compositions of the present invention may comprise humectants which are preferably present at a level of from about 0.01% to about 20%, preferably from about 0.1% to about 15% and preferably from about 0.5% to about 10%. Preferred humectants include, but are not limited to, compounds selected from polyhydric alcohols, sorbitol, glycerol, urea, betaine, D or DL panthenol, calcium pantothenate, royal jelly, panthetine, pantotheine, panthenyl ethyl ether, pangamic acid, pyridoxin, pantoyl lactose Vitamin B complex, sodium pyrrolidone carboxylic acid, hexane - 1, 2, 6, - triol, guanidine or its derivatives, and mixtures thereof.

Suitable polyhydric alcohols for use herein include polyalkylene glycols and preferably alkylene polyols and their derivatives, including propylene glycol, dipropylene glycol, polypropylene glycol, polyethylene glycol and derivatives thereof, sorbitol, hydroxypropyl sorbitol, erythritol, threitol, pentaerythritol, xylitol, glucitol, mannitol, pentylene glycol, hexylene glycol, butylene glycol (e.g., 1,3-butylene glycol), hexane triol (e.g., 1,2,6-hexanetriol), trimethylol propane, neopentyl glycol, glycerine, ethoxylated glycerine, propane-1,3 diol, propoxylated glycerine and mixtures thereof. The alkoxylated derivatives of any of the above polyhydric alcohols are also suitable for use herein. Preferred polyhydric alcohols of the present invention are selected from glycerine, butylene glycol, propylene glycol, pentylene glycol, hexylene glycol, dipropylene glycol,

polyethylene glycol, hexane triol, ethoxylated glycerine and propoxylated glycerine and mixtures thereof.

Suitable humectants useful herein are sodium 2-pyrrolidone-5-carboxylate (NaPCA), guanidine; glycolic acid and glycolate salts (e.g. ammonium and quaternary alkyl ammonium); lactic acid and lactate salts (e.g. ammonium and quaternary alkyl ammonium); aloe vera in any of its variety of forms (e.g., aloe vera gel); hyaluronic acid and derivatives thereof (e.g., salt derivatives such as sodium hyaluronate); lactamide monoethanolamine; acetamide monoethanolamine; urea; betaine, panthenol and derivatives thereof; and mixtures thereof.

At least part (up to about 5% by weight of composition) of a humectant can be incorporated in the form of an admixture with a particulate cross-linked hydrophobic acrylate or methacrylate copolymer, itself preferably present in an amount of from about 0.1% to about 10%, which can be added either to the aqueous or disperse phase. This copolymer is particularly valuable for reducing shine and controlling oil while helping to provide effective moisturization benefits and is described in further detail by WO96/03964, incorporated herein by reference.

The oil-in-water and water-in-oil emulsion embodiments of the present invention may comprise from about 0.05% to about 20%, preferably from about 1% to about 15%, preferably from about 2% to about 10%, preferably from about 2% to about 5% of a dermatologically acceptable emollient. Emollients tend to lubricate the skin, increase the smoothness and suppleness of the skin, prevent or relieve dryness of the skin and/or protect the skin. Emollients are typically water-immiscible, oily or waxy materials and emollients can confer aesthetic properties to a topical composition. A wide variety of suitable emollients are known and may be used herein. Sagarin, Cosmetics, Science and Technology, 2nd Edition, Vol. 1, pp. 32-43 (1972), contains numerous examples of materials suitable as an emollient. All emollients discussed in application WO 00/24372 should be considered as suitable for use in the present invention although preferred examples are outlined in further detail below:

i) Straight and branched chain hydrocarbons having from about 7 to about 40 carbon atoms, such as mineral oils, dodecane, squalane, cholesterol, hydrogenated

polyisobutylene, isohexadecane, isoeicosane, isooctahexacontane, isohexapentacontahectane, and the C_7-C_{40} isoparaffins, which are C_7-C_{40} branched hydrocarbons. Suitable branched chain hydrocarbons for use herein are selected from isopentacontaoctactane, petrolatum and mixtures thereof.

ii) C_1-C_{30} fatty acid esters of C_1-C_{30} carboxylic acids, C_{12-15} alkyl benzoates and of C_2-C_{30} dicarboxylic acids, e.g. isononyl isononanoate, isostearyl neopentanoate, isodecyl octanoate, isodecyl isononanoate, tridecyl isononanoate, myristyl octanoate, octyl pelargonate, octyl isononanoate, myristyl myristate, myristyl neopentanoate, myristyl octanoate, isopropyl myristate, myristyl propionate, isopropyl stearate, isopropyl isostearate, methyl isostearate, behenyl behenate, dioctyl maleate, diisopropyl adipate, and diisopropyl dilinoleate and mixtures thereof.

iii) C_1-C_{30} mono- and poly- esters of sugars and related materials. These esters are derived from a sugar or polyol moiety and one or more carboxylic acid moieties. Depending on the constituent acid and sugar, these esters can be in either liquid or solid form at room temperature. Examples include: glucose tetraoleate, the galactose tetraesters of oleic acid, the sorbitol tetraoleate, sucrose tetraoleate, sucrose pentaoleate, sucrose hexaoleate, sucrose heptaoleate, sucrose octaoleate, sorbitol hexaester. Other materials include cottonseed oil or soybean oil fatty acid esters of sucrose. Other examples of such materials are described in WO 96/16636, incorporated by reference herein.

iv) Vegetable oils and hydrogenated vegetable oils. Examples of vegetable oils and hydrogenated vegetable oils include safflower oil, grapeseed oil, coconut oil, cottonseed oil, menhaden oil, palm kernel oil, palm oil, peanut oil, soybean oil, rapeseed oil, linseed oil, rice bran oil, pine oil, nut oil, sesame oil, sunflower seed oil, partially and fully hydrogenated oils from the foregoing sources and mixtures thereof

v) Soluble or colloidally-soluble moisturizing agents. Examples include hyaluronic acid and chondroitin sulfate..

Compositions herein may contain an emulsifier and/or surfactant, generally to help disperse and suspend the disperse phase within the continuous aqueous phase. A surfactant

may also be useful if the product is intended for skin or hair cleansing. For convenience hereinafter emulsifiers will be referred to under the term 'surfactants', thus 'surfactant(s)' will be used to refer to surface active agents whether used as emulsifiers or for other surfactant purposes such as skin cleansing. Known or conventional surfactants can be used in the composition, provided that the selected agent is chemically and physically compatible with essential components of the composition and provides the desired characteristics. Suitable surfactants include non-silicone derived materials, silicone-derived materials, and mixtures thereof. All surfactants discussed in application WO 00/24372 should be considered as suitable for use in the present invention.

The compositions of the present invention may comprise preferably from about 0.05% to about 30%, preferably from about 0.5% to 15%, preferably from about 1% to 10% of a surfactant or mixture of surfactants. The exact surfactant or surfactant mixture chosen will depend upon the pH of the composition, the other components present and the desired final product aesthetics.

Among the nonionic surfactants that are useful herein are those that can be broadly defined as condensation products of long chain alcohols, e.g. C_{8-30} alcohols, with sugar or starch polymers ie glycosides. Other useful nonionic surfactants include the condensation products of alkylene oxides with fatty acids (i.e. alkylene oxide esters of fatty acids). These materials have the general formula $RCO(X)_nOH$ wherein R is a C_{10-30} alkyl group, X is $-OCH_2CH_2-$ (i.e. derived from ethylene glycol or oxide) or $-OCH_2CHCH_3-$ (i.e. derived from propylene glycol or oxide) and n is an integer from about 6 to about 200. Other nonionic surfactants are the condensation products of alkylene oxides with 2 moles of fatty acids (i.e. alkylene oxide diesters of fatty acids). These materials have the general formula $RCO(X)_nOOCR$ wherein R is a C_{10-30} alkyl group, X is $-OCH_2CH_2-$(i.e. derived from ethylene glycol or oxide) or $-OCH_2CHCH_3-$(i.e. derived from propylene glycol or oxide) and n is an integer from about 6 to about 100. An emulsifier for use herein is preferably a fatty acid ester blend based on a mixture of (for example) sorbitan fatty acid ester and sucrose fatty acid ester, especially (TOM: preferably?) a blend of sorbitan stearate and sucrose cocoate. Even further suitable examples include a mixture of cetearyl alcohols and cetearyl glucosides.

The hydrophilic surfactants useful herein can alternatively or additionally include any of a wide variety of cationic, anionic, zwitterionic, and amphoteric surfactants such as are known in the art. (See, e.g., McCutcheon's, Emulsifiers and Detergents, North American and International Editions (2003), published by MC Publishing Co. ; U.S. Patent No. 5,011,681 to Ciotti et al., issued April 30, 1991; U.S. Patent No. 4,421,769 to Dixon et al., issued December 20, 1983; and U.S. Patent No. 3,755,560 to Dickert et al., issued August 28, 1973).

A variety of anionic surfactants are also useful herein. (See, e.g., U.S. Patent No. 3,929,678, to Laughlin et al., issued December 30, 1975). Exemplary anionic surfactants include the alkoyl isethionates (e.g., C_{12} - C_{30}), alkyl and alkyl ether sulfates and salts thereof, alkyl and alkyl ether phosphates and salts thereof, alkyl methyl taurates (e.g., C_{12} - C_{30}), and soaps (e.g., substituted alkylamine and alkali metal salts, e.g., sodium or potassium salts) of fatty acids.

Amphoteric and zwitterionic surfactants are also useful herein. Examples of amphoteric and zwitterionic surfactants which can be used in the compositions of the present invention are those which are broadly described as derivatives of aliphatic secondary and tertiary amines in which the aliphatic radical can be straight or branched chain and wherein one of the aliphatic substituents contains from about 8 to about 22 carbon atoms (preferably C_8 - C_{18}) and one contains an anionic water solubilizing group, e.g., carboxy, sulfonate, sulfate, phosphate, or phosphonate. Examples are alkyl imino acetates and iminodialkanoates and aminoalkanoates, imidazolinium and ammonium derivatives. Other suitable amphoteric and zwitterionic surfactants are those selected from the group consisting of betaines, sultaines, hydroxysultaines, and branched and unbranched alkanoyl sarcosinates, and mixtures thereof.

Some emulsions of the present invention may include a silicone containing emulsifier or surfactant. A wide variety of silicone emulsifiers are useful herein. These silicone emulsifiers are typically organically modified organopolysiloxanes, also known to those skilled in the art as silicone surfactants. Useful silicone emulsifiers include dimethicone copolyols. These materials are polydimethyl siloxanes which have been modified to include polyether side chains such as polyethylene oxide chains, polypropylene

oxide chains, mixtures of these chains and polyether chains containing moieties derived from both ethylene oxide and propylene oxide. Other examples include alkyl-modified dimethicone copolyols, i.e., compounds which contain C_2-C_{30} pendant side chains. Still other useful dimethicone copolyols include materials having various cationic, anionic, amphoteric, and zwitterionic pendant moieties.

The compositions of the present invention can comprise at least one polymeric thickening agent. The polymeric thickening agents useful herein preferably have a number average molecular weight of greater than about 20,000, preferably greater than about 50,000 and preferably greater than about 100,000. The compositions of the present invention may comprise from about 0.01% to about 10%, preferably from about 0.1% to about 8% and preferably from about 0.2% to about 5% by weight of the composition of the polymeric thickening agent or mixtures thereof.

Preferred polymer thickening agents for use herein include non-ionic thickening agents and anionic thickening agents or mixtures thereof. Suitable non-ionic thickening agents include polyacrylamide polymers, crosslinked poly(N-vinylpyrrolidones), polysaccharides, natural or synthetic gums, polyvinylpyrrolidone and polyvinylalcohol. Suitable anionic thickening agents include acrylic acid/ethyl acrylate copolymers, carboxyvinyl polymers and crosslinked copolymers of alkyl vinyl ethers and maleic anhydride. As an example, Noveon sells a thickener under the trade mark of Carbopol resins or mixtures thereof. Suitable Carbopol resins may be hydrophobically modified, and other suitable resins are described in WO98/22085, or mixtures thereof.

The present compositions may comprise at least one silicone oil phase. Silicone oil phase(s) generally comprises from about 0.1% to about 20%, preferably from about 0.5% to about 10%, preferably from about 0.5% to about 5%, of the composition. The silicone oil phase preferably comprises one or more silicone components.

Silicone components can be fluids, including straight chain, branched and cyclic silicones. Suitable silicone fluids useful herein include silicones inclusive of polyalkyl siloxane fluids, polyaryl siloxane fluids, cyclic and linear polyalkylsiloxanes, poly-alkoxylated silicones, amino and quaternary ammonium modified silicones, polyalkylaryl siloxanes or a polyether siloxane copolymer and mixtures thereof. The silicone fluids can

be volatile or non-volatile. Silicone fluids generally have an average molecular weight of less than about 200,000. Suitable silicone fluids have a molecular weight of about 100,000 or less, preferably about 50,000 or less, preferably about 10,000 or less. Preferably the silicone fluid is selected from silicone fluids having a weight average molecular weight in the range from about 100 to about 50,000 and preferably from about 200 to about 40,000. Typically, silicone fluids have a viscosity ranging from about 0.65 to about 600,000 mm^2s^{-1}, preferably from about 0.65 to about 10,000 $mm^2.s^{-1}$ at 25°C. The viscosity can be measured by means of a glass capillary viscometer as set forth in Dow Corning Corporate Test Method CTM0004, July 29, 1970. Suitable polydimethyl siloxanes that can be used herein include those available, for example, from the General Electric Company and from Dow Corning. Also useful are essentially non-volatile polyalkylarylsiloxanes, for example, polymethylphenylsiloxanes, having viscosities of about 0.65 to 30,000 mm^2s^{-1} at 25°C. These siloxanes are available, for example, from the General Electric Company or from Dow Corning. Cyclic polydimethylsiloxanes suitable for use herein are those having a ring structure incorporating from about 3 to about 7 $(CH_3)_2SiO$ moieties, preferably about 5 or more.

Silicone gums can also be used herein. In preferred embodiments, a silicone oil phase comprises a silicone gum or a mixture of silicones including the silicone gum. Typically, silicone gums have a viscosity at 25°C in excess of about 1,000,000 mm^2s^{-1}. The silicone gums include dimethicones as described by Petrarch and others including US-A-4,152,416, May 1, 1979 to Spitzer, et al, and Noll, Walter, Chemistry and Technology of Silicones, New York: Academic Press 1968. Also describing silicone gums are General Electric Silicone Rubber Product Data Sheets SE 30, SE 33, SE 54 and SE 76. Specific examples of silicone gums include polydimethylsiloxane, (polydimethylsiloxane)-(methylvinylsiloxane) copolymer, poly(dimethylsiloxane)(diphenyl)(methylvinylsiloxane) copolymer and mixtures thereof. Preferred silicone gums for use herein are silicone gums having a molecular weight of from about 200,000 to about 4,000,000 selected from dimethiconol, dimethicone copolyol, dimethicone and mixtures thereof.

A silicone phase herein preferably comprises a silicone gum incorporated into the composition as part of a silicone gum-fluid blend. When the silicone gum is incorporated

as part of a silicone gum-fluid blend, the silicone gum preferably constitutes from about 5% to about 40%, especially from about 10% to 20% by weight of the silicone gum-fluid blend. Suitable silicone gum-fluid blends herein are mixtures consisting essentially of:

(i) a silicone having a molecular weight of from about 200,000 to about 4,000,000 selected from dimethiconol, fluorosilicone and dimethicone and mixtures thereof; and

(ii) a carrier which is a silicone fluid, the carrier having a viscosity from about 0.65 mm^2s^{-1} to about 100 mm^2s^{-1},

wherein the ratio of i) to ii) is from about 10:90 to about 20:80 and wherein said silicone gum-based component has a final viscosity of from about 100 mm^2s^{-1} to about 100,000 mm^2s^{-1}, preferably from 500 mm^2s^{-1} to about 10,000 mm^2s^{-1}.

Further silicone components suitable for use in a silicone oil phase herein are crosslinked polyorganosiloxane polymers, optionally dispersed in a fluid carrier. In general, when present the crosslinked polyorganosiloxane polymers, together with its carrier (if present) comprises from about 0.1% to about 20%, preferably from about 0.5% to about 10%, preferably from about 0.5% to about 5% of the composition. Such polymers comprise polyorganosiloxane polymers crosslinked by a crosslinking agent. Suitable crosslinking agents are disclosed in WO98/22085. Examples of suitable polyorganosiloxane polymers for use herein include methyl vinyl dimethicone, methyl vinyl diphenyl dimethicone and methyl vinyl phenyl methyl diphenyl dimethicone.

Another class of silicone components suitable for use in a silicone oil phase herein includes polydiorganosiloxane-polyoxyalkylene copolymers containing at least one polydiorganosiloxane segment and at least one polyoxyalkylene segment. Suitable polydiorganosiloxane segments and copolymers thereof are disclosed in WO98/22085. Suitable polydiorganosiloxane-polyalkylene copolymers are available commercially under the tradenames Belsil (RTM) from Wacker-Chemie GmbH. A particularly preferred copolymer fluid blend for use herein includes Dow Corning DC3225C which has the CTFA designation Dimethicone/Dimethicone copolyol.

Compositions of the present invention may comprise an organic sunscreen. Suitable sunscreens can have UVA absorbing properties, UVB absorbing properties or a mixture thereof. The exact amount of the sunscreen active will vary depending upon the desired Sun Protection Factor, ie the "SPF" of the composition as well as the desired level of UV protection. SPF is a commonly used measure of photoprotection of a sunscreen against erythema. The SPF is defined as a ratio of the ultraviolet energy required to produce minimal erythema on protected skin to that required to produce the same minimal erythema on unprotected skin in the same individual. Amounts of the sunscreen used are typically (TOM: preferably?) from about 2% to about 20%, more typically (TOM: preferably?) from about 4% to about 14%. Suitable sunscreens include, but are not limited to those approved for use in the United States, Japan, Europe and Australia. The compositions of the present invention preferably comprise an SPF of about 2 to about 30, preferably about 4 about 30, preferably about 4 to about 15.

The compositions of the present invention may include one or more UVA absorbing sunscreen actives that absorb UV radiation having a wavelength of from about 320nm to about 400nm. Suitable UVA absorbing sunscreen actives are selected from dibenzoylmethane derivatives, anthranilate derivatives such as methylanthranilate and homomethyl, 1-N-acetylanthranilate, and mixtures thereof. Examples of dibenzoylmethane sunscreen actives are described in Sunscreens: Development, Evaluation, and Regulatory Aspects edited by N. J. Lowe and N. A. Shaath, Marcel Dekker, Inc. The UVA absorbing sunscreen active is preferably present in an amount to provide broad spectrum UVA protection either independently, or in combination with, other UV protective actives which may be present in the composition.

Suitable UVA sunscreen actives are dibenzoylmethane sunscreen actives and their derivatives. They include, but are not limited to, those selected from 2-methyldibenzoylmethane, 4-methyldibenzoylmethane, 4-isopropyldibenzoylmethane, 4-tert-butyldibenzoylmethane, 2, 4-dimethyldibenzoylmethane, 2, 5-dimethyldibenzoylmethane, 4, 4'-diisopropylbenzoylmethane, 4-(1, 1-dimethylethyl)-4'-methoxydibenzoylmethane, 2-methyl-5-isopropyl-4'-methoxydibenzoylmethane, 2-methyl-5-tert-butyl-4'-methoxy-dibenzoylmethane, 2, 4-dimethyl-4'-methoxydibenzoylmethane, 2, 6-dimethyl-4'-tert-butyl-4'methoxydibenzoylmethane, and mixtures thereof. Preferred dibenzoyl sunscreen actives

include those selected from 4-(1, 1-dimethylethyl)-4'-methoxydibenzoylmethane, 4-isopropyldibenzoylmethane, and mixtures thereof. A preferred sunscreen active is 4-(1, 1-dimethylethyl)-4'-methoxydibenzoylmethane.

The sunscreen active 4-(1, 1-dimethylethyl)-4'-methoxydibenzoylmethane, which is also known as butyl methoxydibenzoylmethane or Avobenzone, is commercially available under the names of Parsol® 1789 from Givaudan Roure (International) S. A. and Eusolex® 9020 from Merck & Co., Inc. The sunscreen 4-isoproplydibenzoylmethane, which is also known as isopropyldibenzoylmethane, is commercially available from Merck under the name of Eusolex® 8020.

The compositions of the present invention may further include one or more UVB sunscreen actives that absorbs UV radiation having a wavelength of about 290nm to about 320nm. The compositions comprise an amount of the UVB sunscreen active that is safe and effective to provide UVB protection either independently, or in combination with, other UV protective actives which may be present in the compositions. The compositions may comprise from about 0.1% to about 20%, preferably from about 0.1% to about 12%, and preferably from about 0.5% to about 8% by weight, of each UVB absorbing organic sunscreen, or as mandated by the relevant regulatory authority(s).

A variety of UVB sunscreen actives are suitable for use herein. Non-limiting examples of such organic sunscreen actives are described in US Patent No 5,087,372 issued February 11, 1992 to Haffey et al.; and US Patent Nos 5,073,371 and 5,073,372 both issued on December 17, 1991 to Turner et al. Still other useful sunscreens are those disclosed in U.S. Patent No. 4,937,370, to Sabatelli, issued June 26, 1990; and U.S. Patent No. 4,999,186, to Sabatelli et al., issued March 12, 1991. Preferred UVB sunscreen actives are selected from 2-ethylhexyl-2-cyano-3, 2-ethylhexyl N,N-dimethyl-p-aminobenzoate, p-aminobenzoic acid, oxybenzone, homomenthyl salicylate, octyl salicylate, 4,4'-methoxy-t-butyldibenzoylmethane, 4-isopropyl dibenzoylmethane, 3-benzylidene camphor, 3-(4-methylbenzylidene) camphor, 3 -diphenylacrylate, 2-phenyl-benzimidazole-5-sulphonic acid (PBSA), cinnamate esters and their derivatives such as 2-ethylhexyl-p-methoxycinnamate, salicylate esters and their derivatives such as triethanolamine salicylate, ethylhexyl salicylate, octyldimethyl para-aminobenzoic acid, camphor derivatives and their derivatives, and mixtures thereof. Preferred organic sunscreen actives are 2-

ethylhexyl-2-cyano-3, 3-diphenylacrylate, 2-phenyl- benzimidazole-5-sulphonic acid (PBSA), octyl-p-methoxycinnamate, and mixtures thereof. Salt and acid neutralized forms of the acidic sunscreens are also useful herein.

An agent may also be added to any of the compositions useful in the present invention to stabilize the UVA sunscreen to prevent it from photo-degrading on exposure to UV radiation and thereby maintaining its UVA protection efficacy. A wide range of compounds have been cited as providing these stabilizing properties and should be chosen to compliment both the UVA sunscreen and the composition as a whole. Suitable stabilizing agents include, but are not limited to, those described in US Patents Nos 5,972,316; 5,968,485; 5,935,556; 5,827,508 and Patent WO 00/06110. Preferred examples of stabilizing agents for use in the present invention include 2-ethylhexyl-2-cyano-3, 3-diphenylacrylate, ethyl-2-cyano-3, 3-diphenylacrylate, 2-ethylhexyl-3, 3-diphenylacrylate, ethyl-3, 3-bis(4-methoxyphenyl)acrylate, diethylhexyl 2,6 napthalate and mixtures thereof (Symrise Chemical Company).

An agent may also be added to any of the compositions useful in the present invention to improve the skin substantivity of those compositions, particularly to enhance their resistance to being washed off by water or rubbed off. Examples include, but are not limited to, acrylates/C_{12-22} alkylmethacrylate copolymer, acrylate/acrylate copolymer, dimethicone, dimethiconol, graft-copoly (dimethylsiloxane/i-butyl methacrylate), lauryl dimethicone, PVP/Hexadecane copolymer, PVP/Eicosene copolymer, tricontanyl PVP and trimethoxysiloxysilicate.

In addition to the organic sunscreens, compositions of the present invention can additionally comprise inorganic physical sunblocks. Non-limiting examples of suitable physical sunblocks are described in CTFA International Cosmetic Ingredient Dictionary, 6th Edition, 1995, pp. 1026-28 and 1103, Sayre, R. M. et al., "Physical Sunscreens", J. Soc. Cosmet. Chem., vol 41, no 2, pp. 103-109 (1990) and Lowe et al., as per above. Preferred inorganic physical sunblocks are zinc oxide and titanium dioxide and mixtures thereof.

When used, the physical sunblocks are present in an amount such that the present compositions are transparent on the skin (ie non-whitening), preferably from about 0.5% to about 20%, preferably from about 0.5% to about 10%, preferably from about 0.5% to 5% by

weight. When titanium dioxide is used, it can have an anatase, rutile or amorphous structure. Manufacturers of micronized grade titanium dioxide and zinc oxide for sunscreen use include, but are not limited to Tayca Corporation, Uniqema, Shinetsu Chemical Corporation, Kerr-McGee, Nanophase, Nanosource, Sachtleben, Elementis, and BASF Corporation, as well as their distribution agents and those companies that further process the material for sunscreen use. Physical sunblock particles, e.g., titanium dioxide and zinc oxide, can be uncoated or coated with a variety of materials including but not limited to amino acids, aluminum compounds such as alumina, aluminum stearate, aluminum laurate, and the like; carboxylic acids and their salts e.g., stearic acid and its salts; phospholipids, such as lecithin; organic silicone compounds; inorganic silicone compounds such as silica and silicates and mixtures thereof. (TOM: repeat of first sentence in paragraph?)

The composition of the present invention may also include preservatives. Such preservatives include, but are not limited to pentylene glycol, ethylene diamine tetra acetate (also known as EDTA) and its salts, chlorhexidine (and its diacetate, dihydrochloride, digluconate derivatives), 1,1,1-trichloro-2-methyl-2-propanol, parachloro metaxylenol, polyhexamethylenebiguanide hydrochloride, dehydroacetic acid, diazolidinyl urea, 2,4-dichlorobenzyl alcohol, 4,4-dimethyl-1,3-oxazolidine, formaldehyde, glutaraldehyde, dimethylidantoin, imidazolidinyl urea, 5-Chloro-2-methyl-4-isothiazolin-3-one, ortho-phenylphenol, 4-hydroxybenzoic acid (also known as paraben) and its methyl-, ethyl-, propyl-, isopropyl-, butyl-, isobutyl-esters,trichlosan, 2-phenoxyethanol, phenyl mercuric acetate, quaternium-15, salycilate, salicylic acid and its saltssorbic acid and its salts, iodopropanyl butylcarbamate zinc pyrithione, benzyl alcohol, 5-bromo-5nitro-1,3-dioxane, 2-bromo-2-nitropropane-1,3-diol, benzoic acid and its salts, sulfites, bisulfites, and benzalkonium chloride.

A variety of optional ingredients such as neutralizing agents, perfumes and perfume solubilizing agents, and coloring agents, can also be added to the compositions herein. It is preferred that any additional ingredients enhance the skin softness / smoothness benefits of the product. In addition it is preferred that any such ingredients do not negatively impact the aesthetic properties of the product.

Neutralizing agents suitable for use in neutralizing acidic group containing hydrophilic gelling agents herein include sodium hydroxide, potassium hydroxide, ammonium hydroxide, monoethanolamine, diethanolamine, amino methyl propanol, tris-buffer and triethanolamine.

Other optional materials include any of the various functional and/or active ingredients known to those skilled in the art. (See e.g., McCutcheon's Functional Materials, North American and International Editioins, (2003), published by MC Publishing Co.) Non-limiting examples include: keratolytic agents; soluble or colloidally-soluble moisturizing agents such as hyaluronic acid and chondroitin sulfate; vitamins such as vitamin A, vitamin C, vitamin E, vitamin K and derivatives thereof and building blocks thereof; phytantriol; fatty alcohols such as dodecatrienol; alpha and beta hydroxyacids; aloe vera; sphingosines and phytosphingosines, cholesterol; skin whitening agents; N-acetyl cysteine; coloring agents; Examples of alpha hydroxy acids include glycolic acid, lactic acid, malic acid, and citric acid (whether derived synthetically or from natural sources and whether used alone or in combination) and their esters or relevant buffered combinations. Other examples of alpha-hydroxy acids include: alpha-hydroxy ethanoic acid, alpha-hydroxyoctanoic acid, alpha-hydroxycaprylic acid, and hydroxycaprylic acid. Preferred examples of alpha hydroxy acids are glycolic acid and lactic acid. It is preferred that alpha hydroxy acids are used in levels of up to about 10%.

Optional materials include pigments that, where water-insoluble, contribute to and are included in the total level of oil phase ingredients. Pigments suitable for use in the compositions of the present invention can be organic and/or inorganic. Also included within the term pigment are materials having a low color or luster, such as matte finishing agents, light scattering agents, and formulation aids such as micas, seracites, and carbonate salts. Further examples of suitable pigments are titanium dioxide, iron oxides, zinc oxide, bismuth oxychloride (whether pre-dispersed and/or pre-coated or not) D&C dyes and lakes, FD&C colors, natural color additives such as carmine, and mixtures thereof. Depending upon the type of composition, a mixture of pigments will normally be used. Preferred pigments for use herein from the viewpoint of moisturization, skin feel, skin appearance and emulsion compatibility are treated pigments. The pigments can be treated with compounds, including but not limited to amino acids, silicones, lecithin and ester oils.

Suitably, the pH of the compositions herein is in the range from about 3.5 to about 10, preferably from about 4 to about 8, preferably from about 5 to about 7, wherein the pH of the final composition is adjusted by addition of acidic, basic or buffer salts as necessary, depending upon the composition of the forms and the pH-requirements of the compounds.

The compositions of the present invention are prepared by standard techniques well known to those skilled in the art. In general the aqueous phase and/or the oil phase would be prepared separately, with materials of similar phase partitioning being added in any order. If the final product is an emulsion, the two phases will then be combined with vigorous stirring and/or homogenization as necessary to reduce the size of the internal phase droplets. Any ingredients in the formulation with high volatility, or which are susceptible to hydrolysis or decomposition at high temperatures, can be added with gentle stirring towards the end of the process, post emulsification if applicable.

Dosage frequency and amount will depend upon the desired performance criteria.

In a fourth aspect, the invention is drawn to a method of decreasing TGFβ-1 activity, the method comprising applying to an organism in need thereof an effective amount of any one of the compounds set forth herein. The fourth aspect includes applications drawn to hair treatment, as disclosed in the first three aspects, as well as other applications (e.g., wound healing, treatment of proliferative diseases, etc). In preferred embodiments, the fourth aspect includes compounds for treatment of an organism in need thereof.

EXAMPLES

EXAMPLE 1: Dermatological Compositions may be prepared as provided below

Dermatological compositions comprising any of the compounds of the invention may be provided as follows:

MOISTURIZSING BODYWASH **pH = 7**

RAW MATERIAL (INCI Designation)	**Amount**
Deionized Water	**QS**
Glycerin	**4.0**
PEG-6 Caprylic/Capric Glycerides	**4.0**
Palm Kernel Fatty acids	**3.0**
Sodium Laureth-3 Sulphate	**45.0**
Cocamide MEA	**3.0**
Sodium Lauroamphoacetate	**25.0**
Soybean Oil	**10.0**
Polyquaternium-10	**0.70**
Preservative, fragrance, color Compound	QS **1000ppm**

BODY WASH

RAW MATERIAL (INCI Designation)	pH 8 **Amount**	pH 6.5 **Amount**	pH 7 **Amount**
Deionized water	**QS**	**QS**	**QS**
Sodium Laureth Sulphate	**12**	**15**	**8**
Cocamidopropyl Betaine	**8**	**10**	**15**
Decyl Glucoside	**0**	**2**	**1**
Polyquaternium-10	**0.25**	**0**	**0**
Polyquaternium-7	**0**	**0**	**0.7**
Preservative, fragrance, color	QS	QS	QS
Compound	**250ppm**	**500ppm**	**1000ppm**

BODY LOTION

RAW MATERIAL (INCI Designation)	**pH 7** **Amount**	**pH 7** **Amount**	**pH 7.5** **Amount**	**pH 7** **Amount**
Deionized Water	**QS**	**QS**	**QS**	**QS**

Glycerine	**8**	**8**	**10**	**12**
Isohexadecane	**3**	**3**	**3**	**6**
Niacinamide	**0**	**3**	**5**	**6**
IsopropylIsostearate	**3**	**3**	**3**	**3**
Polyacrylamide (and) Isoparaffin (and) Laureth-7	**3**	**3**	**3**	**3**
Petrolatum	**4**	**4**	**4**	**2**
Nylon 12	**2**	**2**	**2.5**	**2.5**
Dimethicone	**2**	**2**	**2.5**	**2.5**
Sucrose Polycottonseed Oil	**1.5**	**1.5**	**1.5**	**1.5**
Stearyl Alcohol 97%	**1**	**1**	**1**	**1**
D Panthenol	**1**	**1**	**1**	**1**
DL-alphaTocopherol Acetate	**1**	**1**	**1**	**1**
Cetyl Alcohol 95%	**0.5**	**0.5**	**0.5**	**1**
Behenyl Alcohol	**1**	**1**	**1**	**0.5**
Cetearyl Alcohol (and) Cetearyl Glucoside	**0.4**	**0.4**	**0.5**	**0.5**
Stearic Acid	**0.15**	**0.15**	**0.15**	**0.15**
PEG-100-Stearate	**0.15**	**0.15**	**0.15**	**0.15**
Preservative, fragrance, color Compounds	QS **250 ppm**	QS **500 ppm**	QS **750 ppm**	QS **1000ppm**

ULTRA-HIGH MOISTURIZSING EMULSION **pH 7** **pH 7**

RAW MATERIAL (INCI Designation)	**Amount**	**Amount**
Deionized water	**QS**	**QS**
Glycerin	**12**	**5**
PEG 400	**0**	**10**
Niacinamide	**5**	**7**
Isohexadecane	**5**	**5**
Dimethicone	**3**	**2**

Polyacrylamide (and) Isoparaffin (and) Laureth-7	**3**	**3**
Isopropyl Isostearate	**2**	**2**
Polymethylsilsesquioxane	**2**	**2**
Cetyl Alcohol 95%	**1**	**1**
Sucrose polycottonseed oil	**1**	**1**
D-Panthenol	**1**	**1**
Tocopherol Acetate	**1**	**1**
Stearyl Alcohol 95%	**0.5**	**0.5**
Cetearyl Glucoside	**0.5**	**0.5**
Titanium dioxide	**0.3**	**0.3**
Stearic Acid	**0.15**	**0.15**
PEG-100-Stearate	**0.15**	**0.15**
Preservative, fragrance, color	QS	QS
Compound	**250 ppm**	**100 ppm**

MOISTURIZING CREAM	**pH 7**	**pH 7**	**pH 7.5**
RAW MATERIAL (INCI Designation)	**Amount**	**Amount**	**Amount**
Deionized water	**QS**	**QS**	**QS**
Glycerine	**3**	**5**	**10**
Petrolatum	**3**	**3**	**0**
Cetyl Alcohol 95%	**1.5**	**1.5**	**1**
Dimethicone Copolyol	**2**	**2**	**2**
Isopropyl Palmitate	**1**	**1**	**0.5**
Carbopol 954 (Noveon)	**0.7**	**0.7**	**0.7**
Dimethicone (350cs)	**1**	**1**	**1**
Stearyl Alcohol 97%	**0.5**	**0.5**	**1**
Stearic acid	**0.1**	**0.1**	**0.1**
Peg-100-stearate	**0.1**	**0.1**	**0.1**
Titanium Dioxide	**0.3**	**0.3**	**0.3**
Preservative, color, fragrance	QS	QS	QS

Compound	50ppm	250ppm	1000ppm

LEAVE-ON HAIR CONDITIONER

RAW MATERIAL (INCI Designation)	**Amount**
Deionized Water	QS
Isostearamidopropyl Morpholine Lactate	6.0
Hydroxyethylcellulose	1.0
Preservative, fragrance, color	QS
Compound	1000ppm

CREAM RINSE **pH 4**

RAW MATERIAL (INCI Designation)	**Amount**
Deionized Water	QS
Behentrimonium Chloride	2.0
Trilaureth-4 Phosphate	1.5
Cetyl alcohol	2.0
Citric acid	QS
Preservative, fragrance, color	QS
Compound	1000ppm

NOURISHING HAIR CONDITIONER /TREATMENT **pH 6**

RAW MATERIAL (INCI Designation)	Amount
Deionized Water	QS
Behentrimonium Methosulfate (and) Cetyl Alcohol	4.0
Wheat germ oil	1.0
Cetyl alcohol	0.5
Propylene glycol	5.0
PEG-60 Lanolin	1.0

Panthenol	2.0
Lupin amino acids	1.0
Cocodimonium Hydroxypropyl Hydrolyzed Wheat Protein	1.0
Fragrance, preservative, color	QS
Compound	1000ppm

CONDITIONING SHAMPOO

RAW MATERIAL (INCI Designation)	Amount
Deionized Water	QS
Sodium Laureth Sulfate 30%	27.0
Cocamidopropyl Betaine	3.7
Coco-Glucoside (and) Glyceryl Oleate	5.0
Coco-Glucoside (and) Glycol Distearate (and) Glycerine	3.0
Guar Hydroxypropyl Trimonium Chloride	0.1
Laureth-2	1.55
Fragrance, preservative, color	QS
Compound	1000ppm

ANTI-DANDRUFF SHAMPOO

RAW MATERIAL (INCI Designation)	Amount
Deionized Water	QS
Magnesium Aluminum Silicate	1.0
Hydroxypropyl Methylcellulose	0.8
Sodium Olefin Sulfate 40%	35.0
Lauramide DEA	4.0
Soyamide DEA	1.0
Quaternium-70 Hydrolyzed Collagen	2.0
Zinc Pyrithione 40%	4.0
Fragrance, preservative, color	QS

Compound	1000ppm

EXAMPLE 2: Panning of a Phage Displayed Peptide Library:

A commercially available phage peptide library PhD C7C (New England Biolabs, Beverly, MA) was panned against TGFβ-1- according to the manufacturers instructions. Phage that was still bound to the target after extensive washes, including an acid wash, was used as a template for a PCR reaction.

EXAMPLE 3: Construction of a peptide-BLA library

PCR product after one round of phage panning was cloned into pME 30.16 to obtain library pGV02-L. pGV02-L encodes 9-amino acid peptide sequences fused to the N-terminus of *Enterobacter cloaceae* ß-lactamase (BLA) with a pIII signal sequence and C-terminal 6XHis tag, (Figure 1). The plasmid also carries a chloramphenicol resistance gene (CAT) as a selectable marker and expression is driven by a lac promoter (Plac). Library pGV02-L was constructed using a BLA vector, pME30.16. pME30.16 was digested with *BbsI* (New England Biolabs, Beverly, MA). To make the inserts the PCR product was used as a template for a PCR reaction using primers with BbsI tails.

Oligos:

ME 190f: GCTATTCAATGTCAGACGAAGACGTCGTTCCTTTCTATTCTCACTCT
ME 190r:
GGTGGAGGTTCGGCGTCTTCCCGACTGAATGGCTAT

The cut vector, and stuffer insert (200bp) were ligated overnight at 16 °C in a 1:5 molar ratio respectively using 10 µl of the DNA mix and 10 ul of Takara solution I ligase. Ligations were purified using Zymo Research DNA clean kit and eluted in 2x 8ul of water. 5 µl of ligation mix was transformed into 40 µl Top 10 electrocompetent cells (Invitrogen), 260 µl SOC was added and the cells grown forl h at 37 °C. The transformation mix was

diluted 1/10 and plated on both LA + 5 ppm CMP and LA + 5 ppm CMP + 0.1 ppm CTX plates, followed by incubation overnight at 37 °C.

EXAMPLE 4: Primary Screen of anti-TGFβ-1-BLA using nitrocefin

Peptides fused to the N-terminus of b-Lactamase (BLA) were screened for binding to TGFβ-1, using enzymatic activity on the lactamase substrate Nitrocefin as the reporter.

Growth and Assay Procedure

Peptide-BLA fusions were expressed in *E. coli*. Growth medium was Luria Broth plus 5 mg/mL chloramphenicol. 100 mL growth medium was added to each well of a sterile Costar 3598 96-well plate (Corning Life Sciences, Corning, NY). One clonal colony was picked into each well using a sterile toothpick, and the plate was covered and placed in a humidified shaker/incubator for 40 hours at 37 degrees C. After incubation, 100 mL "B-PER in Phosphate Buffer" (Pierce Biotechnology, Rockford, IL) was added to each well, and the plate was gently shaken for 30 minutes at room temperature. The resulting cell lysate was diluted 10x in Phosphate Buffered Saline (PBS). 100 mL per well of the diluted cell lysate was added to the assay plate (assay plate preparation is described in the next paragraph), which was then be covered with a plate sealer (Marsh Biosciences, Rochester, NY) and incubated for two hours at room temperature. The assay plate was transferred to a 96-well plate washer (Bio-Tek Instruments, Winooski, VT), where the diluted cell lysate was aspirated and the plate washed once with 100 mL per well, then 3 times with 300 mL PBS plus 0.1% Tween-20 (PBS-T) per well. 200 mL Nitrocefin working solution was immediately added to each well. Nitrocefin working solution will be prepared just prior to use, by diluting a 100 mg/mL (in DMSO) stock 1000-fold in PBS plus 0.125% n-Octyl-Beta-D-Glucopyranoside (Sigma, St. Louis, MO). After addition of the Nitrocefin working solution, the plate was shaken at room temperature for one minute, then read for 5 minutes at 495 nm on a Spectramax plate spectraphotometer (Molecular Devices, Sunnyvale, CA) in kinetic mode. Total BLA activity in the cell lysate was also be measured by diluting cell

lysate 1:100, then combining 20 mL per well of this dilution with 180 mL per well of the Nitrocefin working solution.

Assay Plate Preparation

A Costar 3594 assay plate (Corning Life Sciences, Corning, NY) was prepared by incubating 100 mL per well of a 1 mg/mL solution of TGFβ-1 (R&D Systems, Minneapolis, MN) in 50 mM sodium carbonate, pH 9.6. The plate was covered with a plate sealer (Marsh Biosciences, Rochester, NY) and incubated two hours at room temperature. The plate was transferred to a 96-well plate washer (Bio-Tek Instruments, Winooski, VT), where the TGFβ-1 solution was aspirated and the plate washed 3 times with 300 mL PBS-T per well. "Blocker Casein in PBS" (Pierce Biotechnology, Rockford, IL) will be added at 300 mL per well and the plate was incubated overnight at 4 degrees C. "Blocker Casein in PBS" was aspirated and the plate washed 3 times with 300 mL PBS-T per well. The plate will be used in the assay immediately following the last wash. For screens in which it is important to distinguish specific TGFβ-1 binders from nonspecific (background) binders, a no-target control plate was prepared, beginning with the addition of "Blocker Casein in PBS", and following the same steps from there.

Data Analysis

In the primary screen throughput will be high priority, and the no-target control plate was left out of the procedure. The BLA activity of the bound material was plotted against the total BLA activity in the sample, and points that lie above a y-axis cutoff will be selected as winners. The cutoff is arbitrarily set by the researcher at a value which, for that specific data set, seems likely to separate true binders from the general mass of data points. In the secondary screen, triplicate samples of the "winners" from the primary screen were grown and assayed together, and a no-target control condition was run in parallel, to distinguish TGFβ-1 binders from nonspecific (background) binders. Wild Type BLA were grown and assayed, to give a measure of the natural stickiness of the BLA portion of our fusion molecule. The data was normalized for expression by dividing the TGFβ-1 binding

and background-binding activity by the total BLA activity for every well, and the normalized data sets were plotted against each other (data not shown). Points that lie significantly closer to the "Normalized Bound" axis than the bulk of the data field are selected as winners. Significance is determined by a visual assessment of each point's location in the data field, and the size of its error bars.

The results are shown in Figure 3.

EXAMPLE 5: Secondary Screen of anti-TGFβ-1-BLA using nitrocefin

Individual clones were picked and grown up in 5 ml LB + 5ppm CMP overnight at 37 °C. The cell pastes were then treated with 125 μ of B-PER reagent (Pierce) for 30 min with slow mixing. COSTAR plates (96-well) were coated with 0.1 μg (100 μL of 1 μg/mL) TGFβ-1, carrier-free (R&D Systems) with gentle rocking at 4 °C O/N, followed by blocking with Superblock blocking buffer (Pierce) for several hours at room temperature. 100 ul B-Per treated supernatant from individual clones from pGV02-L were added to the TGFβ-1 coated plates. After one hour, plates were washed six times with PBS, 0.05% Tween 20 and 200 μL of nitrocefin assay buffer containing 0.1 mg/ml nitrocefin (Oxoid, New York) was added to measure residual bound beta-lactamase activity, Abs_{490}/min. Control wells contained pCB04 (*see* Figure 1C) beta-lactamase as a control. Out of 180 clones 3 showed a positive signal. These clones were sequenced, and the results are presented in Table 1, where the first column is the name of the peptide, the second column is the nucleotide sequence and the third column is the polypeptide sequence of the peptide:

Table 1:

1A8	TGTGTGACTACTGATTGGATTGAGTGC	CVTTDWIEC
1E11	TGTTACTATTCGCAATTCCACTAGTCG	CYYSQFHQS
1A12	TGTCCGACGCTGTGGACGCATATGTGCG	CPTLWTHMC

EXAMPLE 6: Purification of 3 sequenced clones

BLA-peptide fusion proteins of the three clones in Example 5 were expressed in *E. coli* (TOP10; Invitrogen) in 15-mL shake flasks in the presence of 5 ppm CMP and 0.1 ppm cefotoxime (CTX) antibiotic at 37 °C overnight. A commercially available purification kit (Insect RoboPop Ni/NTA His Bind Purification Kit, Novagen) was used to purify the 5 peptide BLA fusions according to the manufacturers instructions.

EXAMPLE 7: Cell-Based Assay Using HT-2 Cells:

Biological Activity of the BLA-peptide fusion proteins will be measured in a proliferation bioassay, using HT-2 cells. Assay is well-defined and used by R & D Systems (Minneapolis, MN) as a means in which to measure the activity of cytokines and growth factors. Cells are grown up and then incubated with TGFβ-1 + BLA-peptide fusion or TGFβ-2 + BLA-peptide fusion protein. In order to measure the ability of the BLA-peptide fusion to "neutralize" or antagonize the bioactivity of the TGFβ-1 or TGFβ-2 on HT-2 cells, a constant concentration of TGFβ-1 or TGFβ-2 will be incubated with various concentrations of the peptide for 1 hour at 37°C in a 96-well microtiter plate. Following this preincubation period, the antigen-peptide mixture will be added to cultures of HT-2 cells. The assay mixture will be incubated at 37°C for 44 hours in a CO_2 incubator. 3H-thymidine will be added during the last 4 hours of incubation. The cells are subsequently harvested onto glass fiber filters and the 3H-thymidined incorporated into DNA determined.

EXAMPLE 8: Creation of TGFβ-1/BBI Binding Peptides

TGFβ-1 binding peptides, specifically, binding peptides with inserted peptides as disclosed herein, can be introduced into the BBI trypsin inhibitory loop or chymotrypsin inhibitory loop using the QuikChange II XL Site-Directed Mutagenesis Kit (Stratagene). The mutagenesis reaction can be performed essentially as described in the technical manual provided by the manufacturer (Stratagene). The vector, p2JM103-DNNDPI-BBI, can be used as a template (see Figure 1B). This *B. subtilis* expression vector has the BCE103 cellulase fused to BBI with a cleavable linker between the two domains. Transcription is driven by the *B. subtilis aprE* promoter and the *aprE* signal sequence is included for

secretion of the fusion protein. The vector can be constructed from pUC19 for replication and selection in *E. coli* and also can have an inserted chloramphenicol acetyltransferase gene for selection in *B. subtilis*. In the mutagenesis reaction, approximately 200 ng of the plasmid can be used with 50 pmol of each of the oligonucleotide primers. The reaction mixture can be held at approximately 97 °C for 3 minutes and then held at approximately 50 °C until polymerase is added to each tube. The reaction mixture can then be thermally cycled at 68 °C for 6 minutes, 95 °C for 50 seconds and 55 °C for 50 seconds for 22 times. After cycling, the reaction mixture can be held at 68 °C for 20 minutes and then cooled to 4 °C before continuing on with the protocol. After DpnI digestion, an aliquot of the reaction mixture can be used to transform XL10 Gold cells, colonies picked, plasmids isolated (QIAprep Spin Miniprep Kit, Qiagen), and the variants detected by restriction digests. Positives can be checked for the correct sequence by DNA sequencing.

Example 8 can be seen, for example, in Attorney Docket Numbers GC825P and GC827P, both of which are incorporated by reference, herein.

EXAMPLE 9: BIAcore™ binding analysis: anti-TGFβ-1 peptides

Affinities of the peptides for TGFβ-1 can be measured using a BIAcore™-3000 surface plasmon resonance system (Biacore, Inc., Piscataway, NJ). A CM5 sensor chip will be conditioned with 50 mM NaOH, 0.1% HCl, 0.1% SDS, and 0.08% H_3PO_4 and activated for covalent coupling of FGF-5 using *N*-ethyl-*N*'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions (Biacore, Inc., Piscataway, NJ). Human TGFβ-2, (PeproTech, Rocky Hill, NJ) will be diluted to 5 μg/mL in 10 mM acetic acid, pH 4.0 and injected at a flow rate of 2 μL/min to achieve approximately 1000 to 2000 response units (RU) of coupled protein. An additional solution of EDC and NHS will be injected to improve baseline stability and a solution of 1 M ethanolamine injected as a blocking agent. The reference lane will be activated with EDC and NHS and blocked with ethanolamine.

Peptides will be synthesized using standard FMOC chemistry, purified by reverse phase HPLC to >95% purity (SynPep, Dublin, CA), and will be stored at 10 mg/mL in either water or 10% DMSO. For kinetic measurements, three-fold or four-fold serial

diluted peptides in HBS-EP buffer, 0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20 (Biacore, Inc., Piscataway, NJ), will be injected at 25°C at a flow rate of 50 µL/min. Serial diluted DMSO samples and buffer samples will also be injected for background subtraction. Two 120 sec injections of 20 mM HCl will be used between sample injections for regeneration. Kinetic parameters k_{on}, (M^{-1} sec^{-1}), k_{off} (sec^{-1}) and K_D (M) will be calculated using software programs, Scrubber version 1.1f, BIAevaluation 3.1, and Clamp99 version 3.30.

EXAMPLE 10: *In Vivo* Assay(s)

In-vivo assays can be performed to test each of the constructs for hair care. (See, for example, Tobin et al., Plasticity and Cytokinetic Dynamics of the Hair Follicle Mesenchyme: Implications for Hair Growth Control: Journal of Investigative Dermatology, Volume 120, Issue 6, Page 895, Junee 2003; *see*, also, www.cutech.info).

Having described the preferred embodiments of the present invention, it will appear to those ordinarily skilled in the art that various modifications may be made to the disclosed embodiments, and that such modifications are intended to be within the scope of the present invention.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The molecular complexes and the methods, procedures, treatments, molecules, specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Abstract

The invention is directed to peptides and supported peptides that bind TGFβ-1.

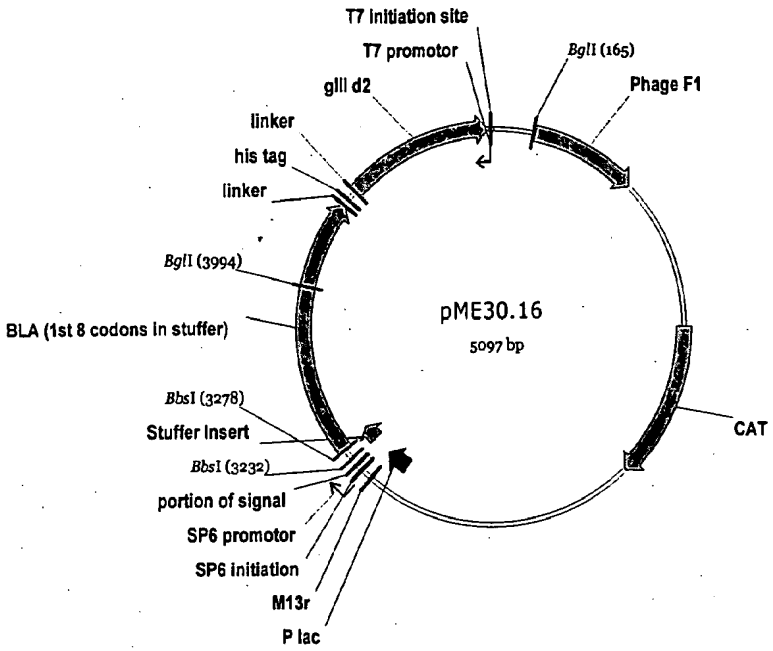
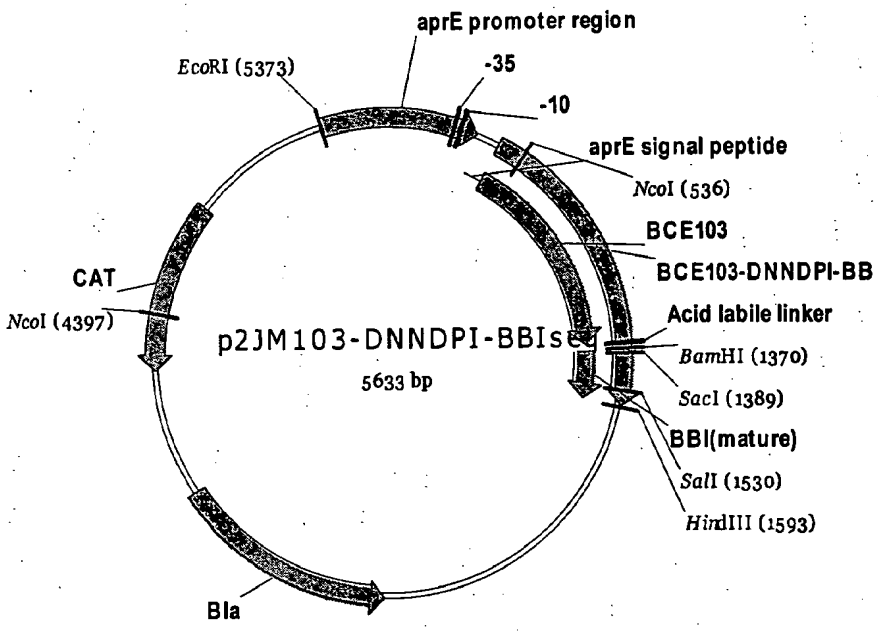

T7 initiation site
T7 promotor
BglI (165)
Phage F1
gIII d2
linker
his tag
linker
BglI (3994)
BLA (1st 8 codons in stuffer)
pME30.16
5097 bp
BbsI (3278)
Stuffer Insert
BbsI (3232)
portion of signal
SP6 promotor
SP6 initiation
M13r
P lac
CAT

FIGURE 1A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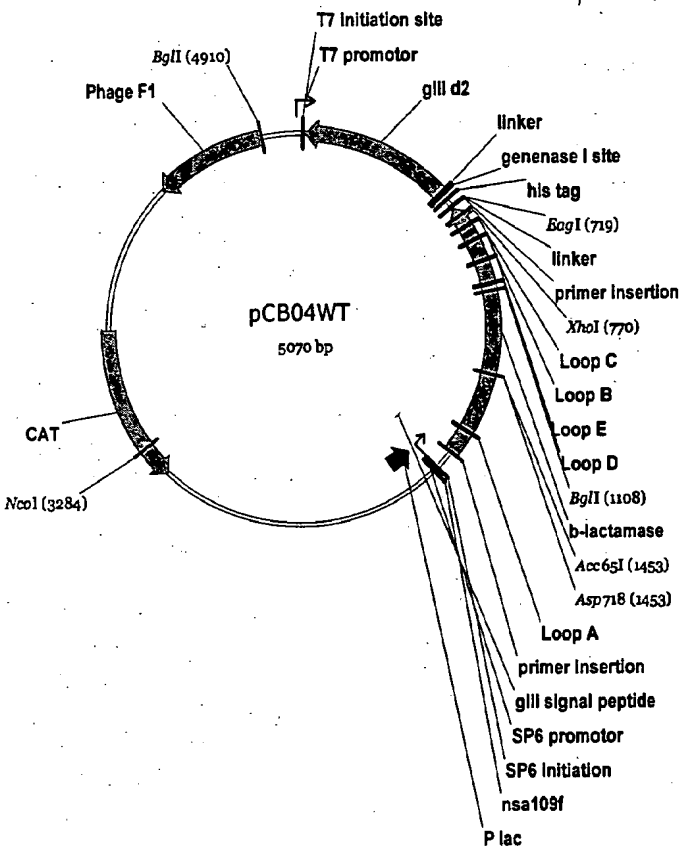
EcoRI (5373)
-35
-10
aprE signal peptide
NcoI (536)
BCE103
BCE103-DNNDPI-BB
Acid labile linker
BamHI (1370)
SacI (1389)
BBI(mature)
SalI (1530)
HindIII (1593)
CAT
NcoI (4397)
p2JM103-DNNDPI-BBIsec
5633 bp
Bla

FIGURE 1B

FIGURE 1C



Gudrun

Figure ~~3A~~ 2: BBI amino acid sequence

DDESSKPCCD QCACTKSNPP QCRCSDMRLN SCHSACKSCI CALSYPAQCF 50
CVDITDFCYE PCKPSEDDKE N 71

TGFb1:

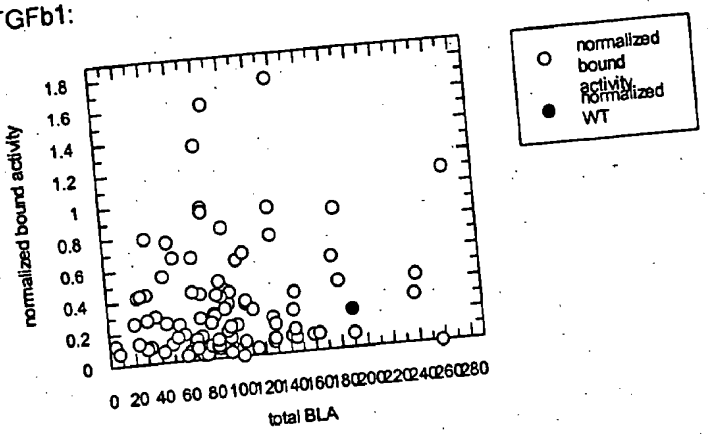


FIGURE 3

TGF Beta-1 SUPPORTED AND BINDING PEPTIDES
Day et al.
SN# Unassigned
Docket No. GC826P
Sheet 5 of 5